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Microtubule-Associated Protein Expression and Predicting Taxane Response

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New Haven, CT 06520-8023

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14. ABSTRACT

We hypothesized that in addition to its predictive value, the microtubule-associated marker tau (MAP-tau) may also function as a prognostic biomarker. The dual functionality of MAP-tau may translate into increased tumor molecular screening information for patients with breast cancer resulting in better treatment options. The results of this work indicate that MAP-tau functions as a prognostic marker for paclitaxel sensitivity when examined using automated quantitative analysis (AQUA) and tissue arrays YTMA 49-5 and YTMA 49-6. Each array contained approximately 750 tumor histospots. This work demonstrates that MAP-tau may be useful for further differentiating ER (+) and ER (-) patients and that increased MAP-tau expression in newly diagnosed breast cancer patients is associated with better outcome. Our findings suggest that MAP-tau may be a useful prognostic marker in addition to its predictive value for taxane response.

15. SUBJECT TERMS
Microtubule-associated proteins (MAPs), MAP-tau, breast cancer, tissue microarrays, biomarkers

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Introduction

Breast cancer is the leading cause of cancer death in women between the ages of 20 and 59 accounts for more than 31% of all new cancers diagnosed in women and is the leading cause of death for women worldwide [1, 2]. While breast cancer family history is an important risk factor, sporadic cases account for more than 90% of all breast cancers and the etiology of this cancer remains largely unknown [3]. Clinical treatment, such as chemotherapy, currently relies on physical examination, imaging, histopathological information, tumor size, lymph node status, degree of metastasis, and biomarker expression (ER, PR, HER2) [4] .

Microtubule stabilizing proteins, such as tau, have begun to gain attention as predictive markers. Tau expression has been found to decrease microtubule vulnerability to taxanes such as *paclitaxel* and its expression makes cells resistant to taxane treatment. Similarly it has recently been shown that low Tau is predictive for response to paclitaxel in breast cancer [5].

Current breast cancer therapy involves the use of taxanes such as paclitaxel and docetaxel [6]. Low tau expression has been shown to be predictive for response to paclitaxel. However, the prognostic value of tau has not been established [5]. This study examined MAP-tau expression in relation to overall patient survival at five years.

Body

In Aim 1 of this project, MAP-tau expression was measured in a large retrospective breast cancer cohort (n=480) with 20 year follow-up using tissue microarray technology and automated quantitative analysis (AQUA). The AQUA system used cytokeratin to define pixels as breast cancer within the array spot, and measured the intensity of tau expression using Cy5 conjugated antibodies. AQUA scores were correlated with clinical and pathologic variables.

MAP-tau showed a normal distribution of expression with high correlation (R= 0.76) between redundant cores. Kaplan-Meier survival analysis with a validated optimal cut-point showed a five year survival rate of 82% for high expressors versus only a 60% survival rate for low expressors (log rank, P<.0001). High tau expression correlated strongly with negative lymph node status (P = 0.0007). Univariate analysis indicated a protective relationship between tau expression and outcome (OR = 0.625, 95% confidence interval [CI] = 0.52-0.75; P<.008).

Task 1: Confirmation of tau as a predictive marker for paclitaxel sensitivity using AQUA

Tau as a predictive marker for paclitaxel sensitivity was confirmed using tissue whole sections and arrays and quantitative analysis was conducted with AQUA.

The following items from the Statement of Work have been **completed:**

a. Order breast test arrays and conduct antibody titration of tau using breast test arrays.
Completed: Tau antibody was titrated using US Biological T1029 mouse monoclonal antibody. Optimal titration: 1:750.

b. Tau antibody staining of the Tissue arrays.
Completed: YTMA 49-5 and YTMA 49-6 were stained with tau T1029 antibody.

c. Image collection, AQUA analysis, and image validation (Appendix A).
Completed. (Fig. 1)

d. AQUA score statistical analysis
Completed (Fig. 2 and Fig. 3; Tables 1, 2, and 3).

Timeline: Months 1-2

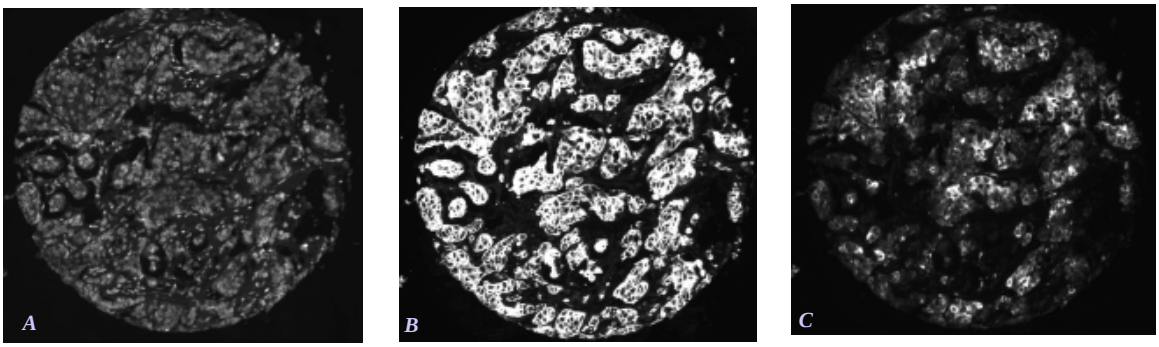


Figure 1. MAP-tau expression was studied in a large retrospective breast cancer cohort (n=656) with long term follow-up using tissue microarray at two-fold redundancy. Automated quantitative analysis (AQUA™) was used for in-situ analysis of protein expression. DAPI was used to define the nuclear region throughout the histospot (A). Cytokeratin was used to define pixels as breast cancer (tumor mask) versus stroma within the histospot (B), and Tau expression was measured using a Cy5-based detection system in the cytoplasm and the nuclear compartments within the tumor mask previously defined by the cytokertin (C). Analysis by AQUA showed a high correlation between cytoplasmic and nuclear tau, so total tau under the mask was used for analysis. AQUA scores for #347: 125.6, 126.7, 136.8 for total tau in tumor mask (shown in C), tau in nuclei and tau in cytoplasmic compartments, respectively (not shown).

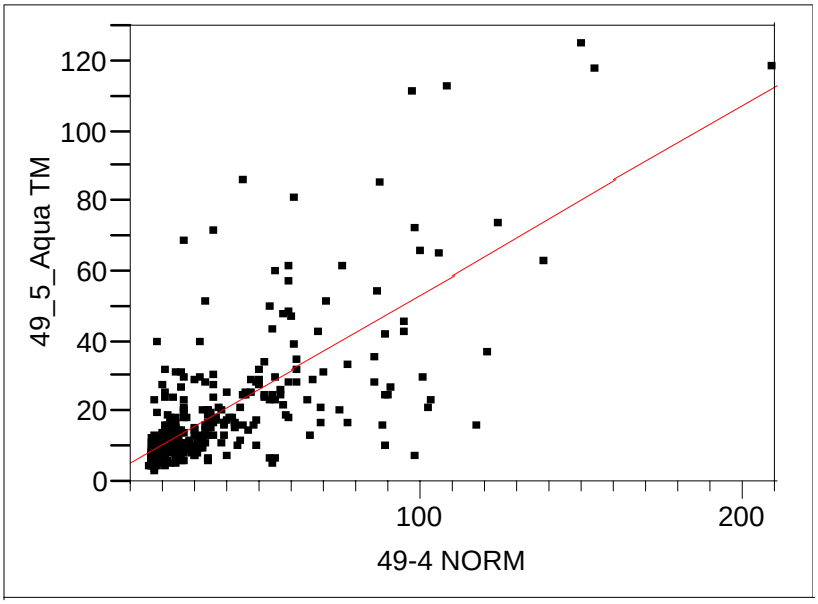


Figure 2. Linear Regression of Microarray YTMA 49-4 and YTMA 49-5 AQUA Scores.

Table 1. Patient Characteristics

Characteristics (n= 656)	
Mean follow-up time	12.8 yrs
Mean age of diagnosis	58.1 yrs
Median follow-up time	8.9 yrs
Median age of diagnosis	58.0 yrs
Censored (20 years) Median follow-up	328 patients 21.4 yrs; 4.2 months minimum
Uncensored (20 years)	276 patients
Node Positive Treatment:	~50% ~15% chemotherapy (Adriamycin, cytoxan, 5-fluorouracil) ~27% tamoxifen (post 1978)
Node Negative Treatment:	~50% Local Radiation and surgical resection only

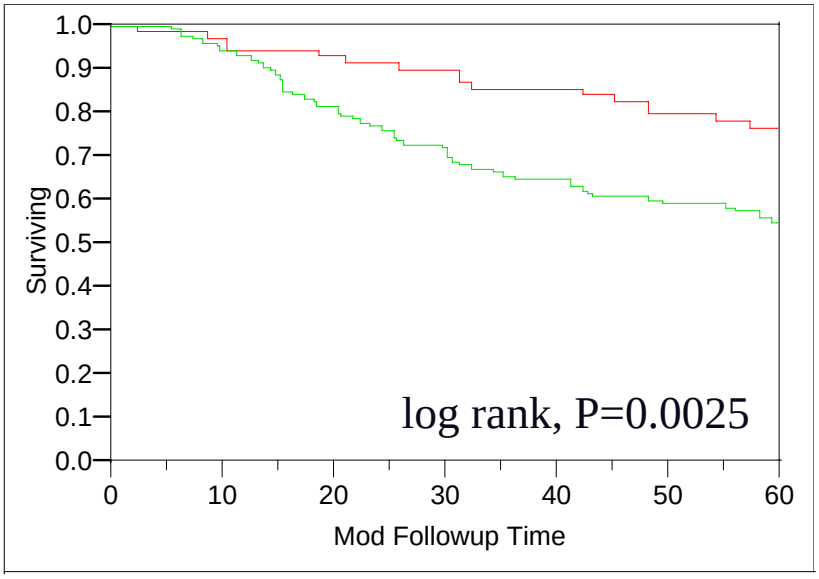
Table 2. Cox Univariate Analysis

Variable	Hazard Ratio
Tau	0.625 (0.51-0.75)

P-value = 0.008

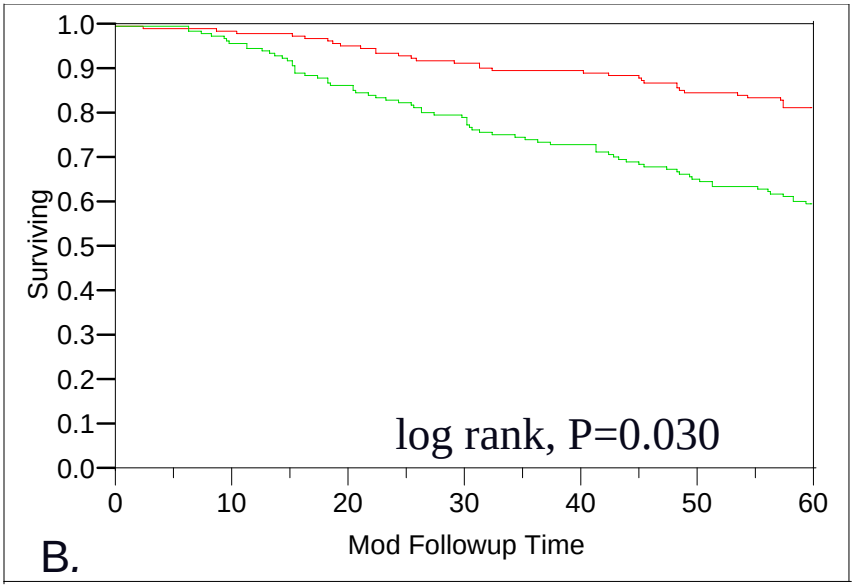
Table 3. Multivariate analysis of tau and histopathologic variables of breast cancer (5-year survival, *n*=364)

Variable	Hazard Ratio	P-Value
Age at diagnosis	1.006 (0.99-1.02)	0.4475
Nodal Status	0.586 (0.45-0.74)	**0.0000**
Total Nodes	0.979 (0.95-1.00)	0.0949
Estrogen Receptor (ER)	0.855 (0.68-1.04)	0.1367
Progesterone Receptor (PR)	0.810 (0.65-0.99)	**0.0416**
HER2	1.235 (1.01-1.43)	**0.0344**
Tumor Size	1.146 (1.06-1.22)	**0.0004**
Tau	0.732 (0.57-0.93)	**0.0112**



Low Tau, ER (-)

A.



High Tau, ER (+)
RED

Low Tau, ER (+)
GREEN

Figure 3. Kaplan-Meier survival analysis for MAP-tau stratified by ER (-) status (panel A) and ER (+) status (panel B).

Task 2: Construction of a Taxane Therapy tissue microarray as a training cohort for future predictive markers beyond tau.

A retrospective cohort of patients treated with taxane therapy will be assembled and tissue samples from this cohort will be used to examine tissue heterogeneity.

The following items from the Statement of Work have been **completed:**

a. Select primary breast carcinoma tumors from the Yale Pathology archives or clinical trials that underwent taxane therapy.
Completed: 140 whole tissue sections were obtained (Appendix B).

b. Design cell line controls for microtubule stabilizing proteins
Completed: 6 YTMA 94-1 microarrays were stained to provide controls for whole tissue sections (Appendix C).

c. Analysis of whole tissue sections and tissue microarrays to examine tissue heterogeneity.
In Progress. 39 of the 140 whole tissue sections have been analyzed (Appendix D and E). Problems with some tissue loss due to whole sections being floated on the slides rather than previous use of tape-transfer method.

Timeline: Months 3-12

Key Research Accomplishments

- This work demonstrated that Tau functions as a prognostic marker for paclitaxel sensitivity using AQUA and the Tissue Arrays YTMA 49-5 and YTMA 49-6.
- MAP-tau may be useful for further differentiating ER (+) and ER (-) patients
- Increased MAP-tau expression is associated with better outcome in breast cancer patients.
- MAP-tau may be a useful prognostic marker in addition to its predictive value for taxane response.
- Examining tissue heterogeneity using both whole tissue sections and tissue microarrays can provide important information regarding the usefulness of tissue microarrays in cancer diagnosis and treatment.

Reportable Outcomes

1. San Antonio Breast Cancer Symposium abstract acceptance and poster presentation San Antonio, Texas. December 2006. (Appendix D).
2. YTMA 49-4 and YTMA 49-5 tissue microarrays stained with T1029 MAP-tau Mab (Appendix A and Fig. 1).
3. Whole Section Tissue database with 15, 604 images. (Appendix E and F)
4. 6 control slides created: YTMA 941 tissue microarray with 120 histospots (Appendix E and F).
5. Data Characterization Algorithm for coding tumor tissue (Appendix E)
6. PhD dissertation research project that is specifically and uniquely breast cancer-focused in Department of Experimental Pathology program at Yale University with mentoring and training emphasis in breast cancer research that would not be possible without this grant.

Conclusion

The current research findings indicate that increased MAP-tau expression is associated with better outcome, that MAP-tau may be useful for further differentiating ER (+) and ER (-) patients, and that MAP-tau may serve as a prognostic marker in addition to its predictive capabilities. The next phase of this project will examine additional microtubule related proteins to compare with MAP-tau.

Our findings may be reflective of increased mitotic arrest and inhibition of cellular proliferation within cancer cells that can occur when high levels of MAP-tau are present. Taxanes function in a similar manner to MAPs by binding and stabilizing microtubules leading to mitotic arrest in cancer cells. Thus, taxanes may be competing for binding sites with tau and this may explain why increased MAP-tau expression results in resistance to taxane treatment (lack of functional binding sites available for paclitaxel) and why low MAP-tau expression is predictive for paclitaxel response (abundance of functional binding sites available for paclitaxel).

The dual functionality of MAP-tau may translate into increased tumor molecular screening information for patients with breast cancer resulting in better treatment options. Consequently, other microtubule associated proteins may also serve as valuable biomarkers for the personalized molecular assessment of breast cancer tumors and we are working to systematically evaluate these proteins.

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2.. Estevez, L.G. & Gradishar, W.J. Evidence-based use of neoadjuvant taxane in operable and inoperable breast cancer. *Clin Cancer Res* **10**, 3249-3261 (2004).
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Appendices

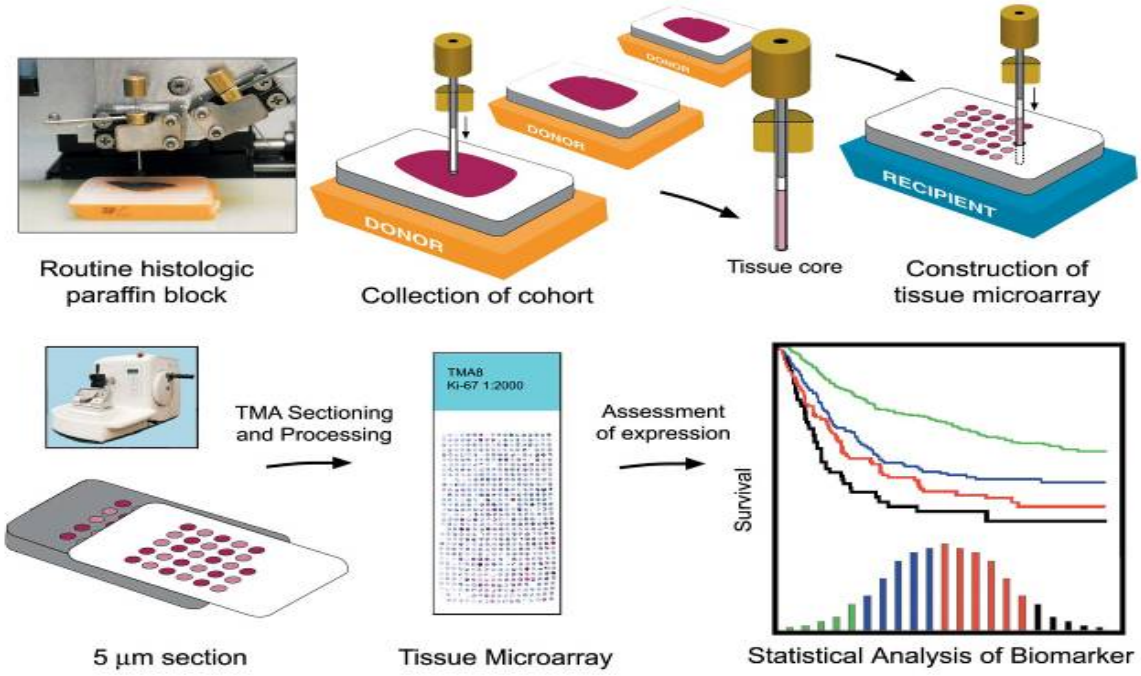
Appendix A

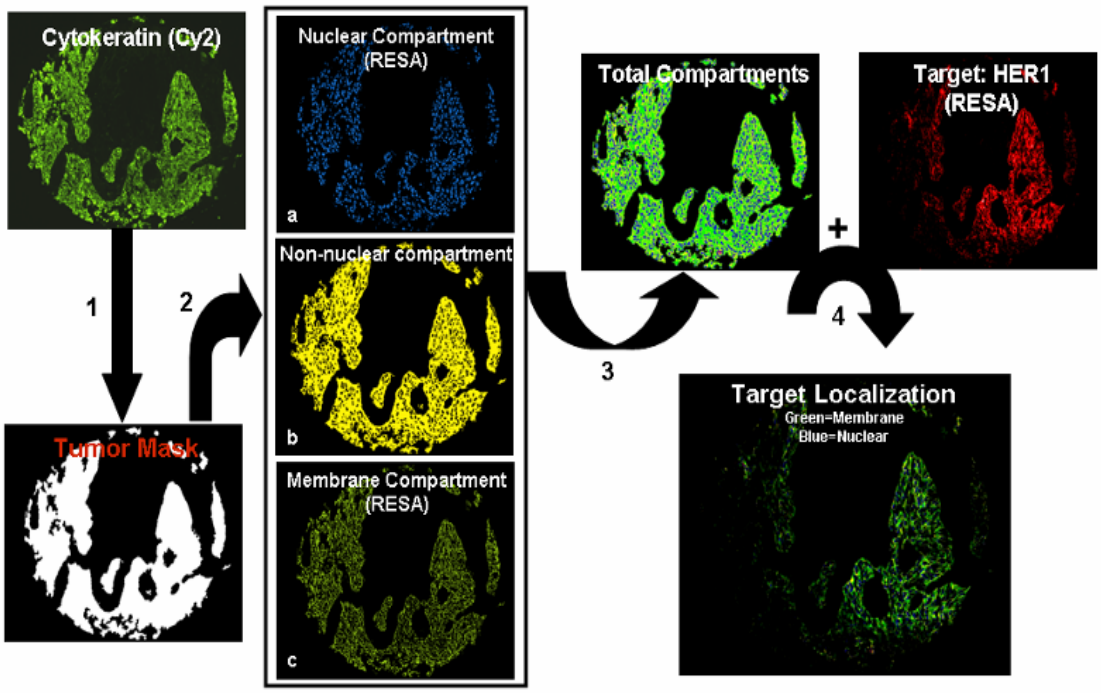
Automated Quantitative Analysis (AQUA)

What is an AQUA Score?

- Each pixel within the mask is assigned a user-defined subcellular compartment (or unassigned)
- The intensity of the "target" of interest is measured on a scale of 0-255 in each pixel in each compartment
- The final score is normalized by dividing the total target intensity by the area of each subcellular compartment.
- The final score is proportional to a number of molecules per unit area.

Methods and Instruments





AQUA Analysis of Tissue

The AQUA software linked to the fluorescence microscopy system allows for quantification
of the protein of interest within the tumor region of each tissue mircroarray core.

Step 1: Cytokeratin is used to separate epithelial tumor from surrounding stroma, creating a tumor mask

Step 2: Different fluorescent tags (like DAPI, Cy-5 tyramide) are used to demarcate subcellular compartments (nuclear, membrane, cytoplasmic, etc).

Step 3: Due to the thickness of the tissue sections and the resulting overlap of compartments, a rapid exponential subtraction algorithm (RESA) is used to subtract an out-of-focus image from an in-focus image, providing improved pixel assignment to subcellular compartments. An AQUA score is generated for each compartment ranging from 0-255 (see box *What is an AQUA score…)*

Step 4: At the Cy-5 wavelength, which is outside the range of tissue autofluroescence, the target of interest is tagged and measured within the subcelluar compartments by the PLACE algorithm.

The resulting AQUA score is the measurement of the biomarker pixel intensity within a compartment divided by the total area of the compartment (to normalize for differences in tumor area in each spot).

Appendix B

TAX 307 Whole Tissue Sections
Patient Characteristics and Study Design

- Cases obtained from the TAX 306 Study Group (2003) (Dr. Lyndsay Harris, Yale Breast Cancer Center)

- Study Design:
  - Multicenter: 58 total in Europe, S. Africa, S. America
    Australia, Canada
  - Randomized (centralized)
  - Non-blinded
  - Phase III

- Objective: compare efficacy & safety AT vs AC as 1st line chemotherapy in 429 patients w/ untreated MBC

  - AT: doxorubicin (DNA intercalation & anthracycline) + docetaxel
  - AC: doxorubicin and cyclophasphamide (alkylating agent)

- Treatment regimen: AT or AC on day 1, every 3 weeks for 8 cycles

- Primary endpoints: Time to treatment progression (TTP)

- Secondary endpoints: overall response rate (ORR), time to treatment failure (TTF), toxicity, survival, quality of life (QoL)

- Inclusion criteria:
  - adjuvant or neoadjuvant <u>non-anthracycline</u> chemo OK
  - prior hormonal therapy OK, but not concurrent
  - NO previous taxanes

- TAX 307 cohort: 140 cases from AT arm

Appendix C

TAX 307 Whole Tissue Sections
Methods

- 140 cases:
  - Floated, whole tumor sections
  - PLUS slides inconsistently used

- 85 matching H&E slides

- 6 control slides: YTMA 94-1; Cell lines for secondary normalization + staining quality control

- Staining:
  - 6 consecutive batches:
  - 25 slides/batch + 1 YTMA 94-1
  - 1 week period: early November 2006

- Target:
  - MAP-tau mouse monocolonal antibody
  - US Biological; 1:750 dilution (titrated)

- Image Capture:
  - HistoRx Image Grabber

- Quantitative analysis of specimens:
  - HistoRx AQUA

Appendix D

29th Annual San Antonio Breast Cancer Symposium

Abstract Number: 551023

Contact/Presenting Author: Maria T. Baquero

Department/Institution: Pathology, Yale Univerisity School of Medicine
Address: 310 Cedar Street/ BML 163, PO Box 208023
City/State/Zip/Country: New Haven, CT, 06520-8023, United States

Phone: 203.671.9926 **Fax:** **E-mail:** mariateresa.baquero@yale.edu

Abstract Categories: 11. Prognostic Factors

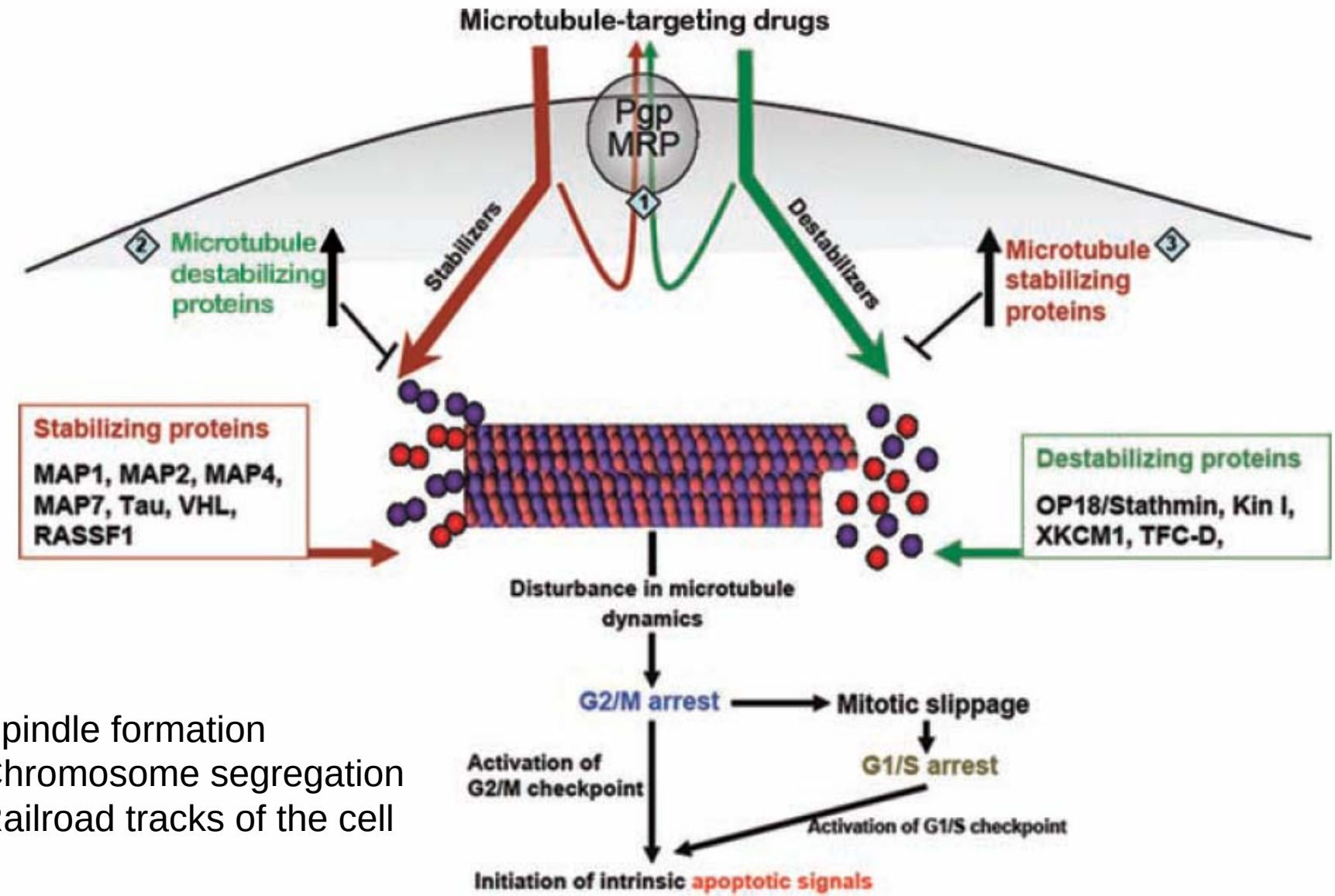
Disclosure: There is no financial interest/arrangement or affiliation with one or more organizations. Off Label: No

Title: Microtubule-associated protein (MAP)-tau is a prognostic biomarker associated with better outcome in breast cancer.

Maria T. Baquero, MPH[1], Mark Gustavson, PhD[1], Jena Giltnane, MS[1], Robert L. Camp, MD, PhD[1] and David L. Rimm, MD, PhD[1]. [1]Department of Pathology, Yale School of Medicine, New Haven, CT, 06520.

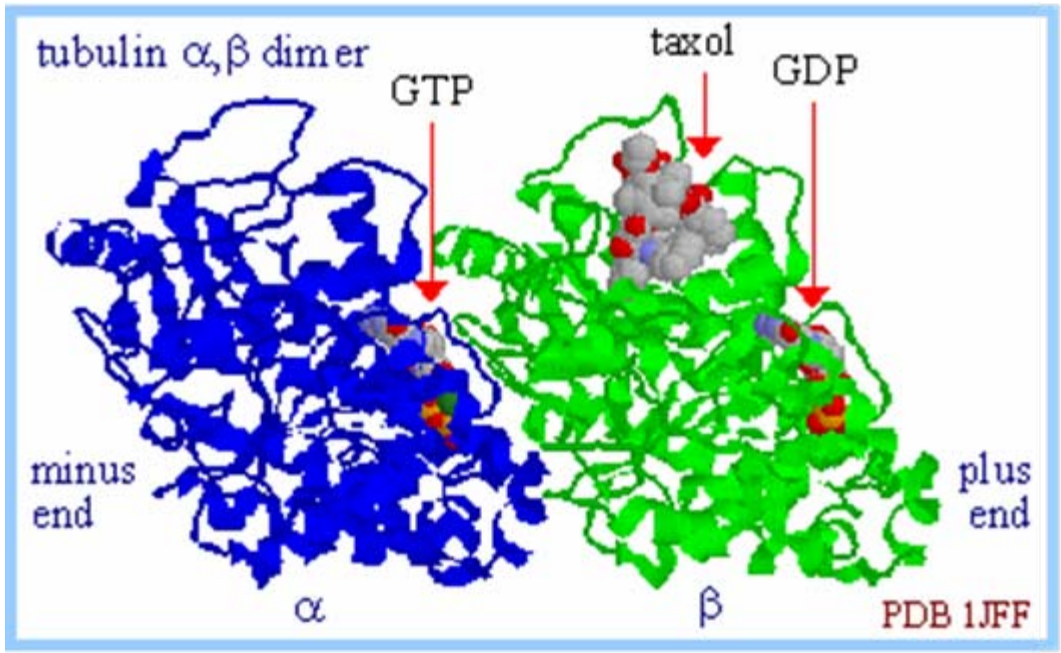
Body: Background: Clinical treatment involving adjuvant or neoadjuvant treatment currently relies on a variety of factors such as tumor size, lymph node status, degree of metastasis, and biomarker expression (ER, PR, HER2). However, additional markers that can identify subsets of patients requiring more aggressive or pathway-targeted adjuvant treatments are needed. Microtubule-associated proteins, such as tau, have recently begun to gain attention as both predictive and prognostic markers. These proteins promote the assembly of tubulin monomers into microtubules functioning to stabilize microtubules and thus working against cancer by inducing mitotic arrest. Tau expression has been found to decrease microtubule vulnerability to taxanes and its expression makes cells resistant to taxane treatment. In addition, low tau expression has been shown to be predictive for response to the taxane, paclitaxel, in breast cancer. However the prognostic value of tau has not been established.
Material and Methods: Tau expression was measured in a large retrospective breast cancer cohort (n=480) with 20 year follow-up using tissue microarray technology and automated quantitative analysis (AQUA). The AQUA system used cytokeratin to define pixels as breast cancer within the array spot, and measured the intensity of tau expression using Cy5 conjugated antibodies. AQUA scores were correlated with clinical and pathologic variables.
Results: Tau showed a normal distribution of expression with high correlation (R= 0.76) between redundant cores. Kaplan-Meier survival analysis with a validated optimal cut-point showed a five year survival rate of 82% for high expressors versus only a 60% survival rate for low expressors (log rank, P<.0001). High tau expression correlated strongly with negative lymph node status (P = 0.0007). Univariate analysis indicated a protective relationship between tau expression and outcome (OR = 0.625, 95% confidence interval [CI] = 0.52-0.75; P<.0001)
Discussion: Similar to microtubule-associated proteins such as tau, taxanes also bind and stabilize microtubules leading to mitotic arrest in cancer cells. Thus, taxanes may compete for binding sites with tau and this may explain why increased tau expression results in resistance to taxane treatment (lack of functional binding sites available for paclitaxel) and why low tau expression is predictive for paclitaxel response (abundance of functional binding sites available for paclitaxel). This study found that increased tau expression is associated with better outcome. This may be reflective of increased mitotic arrest and inhibition of cellular proliferation within cancer cells that can occur when high levels of tau are present. The biological basis of high tau expression and breast cancer pathogenesis requires further investigation. These findings suggest that tau may be a useful prognostic marker in addition to its predictive value in taxane response.

Appendix E



- Spindle formation
- Chromosome segregation
- Railroad tracks of the cell

Kumar M.R. Bhat and Vijayasaradhi Setaluri (2007), AACR



http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb2/part1/microtub.htm

TAX 307 Worksheet Of Images Collected

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H2	27	H12	21	H22	103	H32	52	H42	72
H3	75	H13	121	H23	203	H33	32	H43	77
H4	156	H14	177	H24	41	H34	162	H44	277
H5	161	H15	118	H25	60	H35	96	H45	515
H6	63	H16	107	H26	198	H36	35	H46	b18
H7	41	H17	113	H27	38	H37	50	H47	b99
H8	80	H18	77	H28	36	H38	b15	H48	143
H9	EX	H19	115	H29	75	H39	b22	H49	b22
H10	24	H20	36	H30	304	H40	b28	H50	b64
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H54	EX	H64	262	H74	48	H84	171	H94	137
H55	EX	H65	58	H75	24	H85	20	H95	49
H56	157	H66	407	H76	81	H86	12	H96	325
H57	74	H67	287	H77	10	H87	68	H97	24
H58	8	H68	140	H78	17	H88	EX	H98	126
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H106	109	H116	101	H126	27	H136	168
H107	112	H117	313	H127	24	H137	212
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H109	18	H119	EX	H129	176	H139	171
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TAX 307 Image Worksheet

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		1113	918	1627	1573	.....
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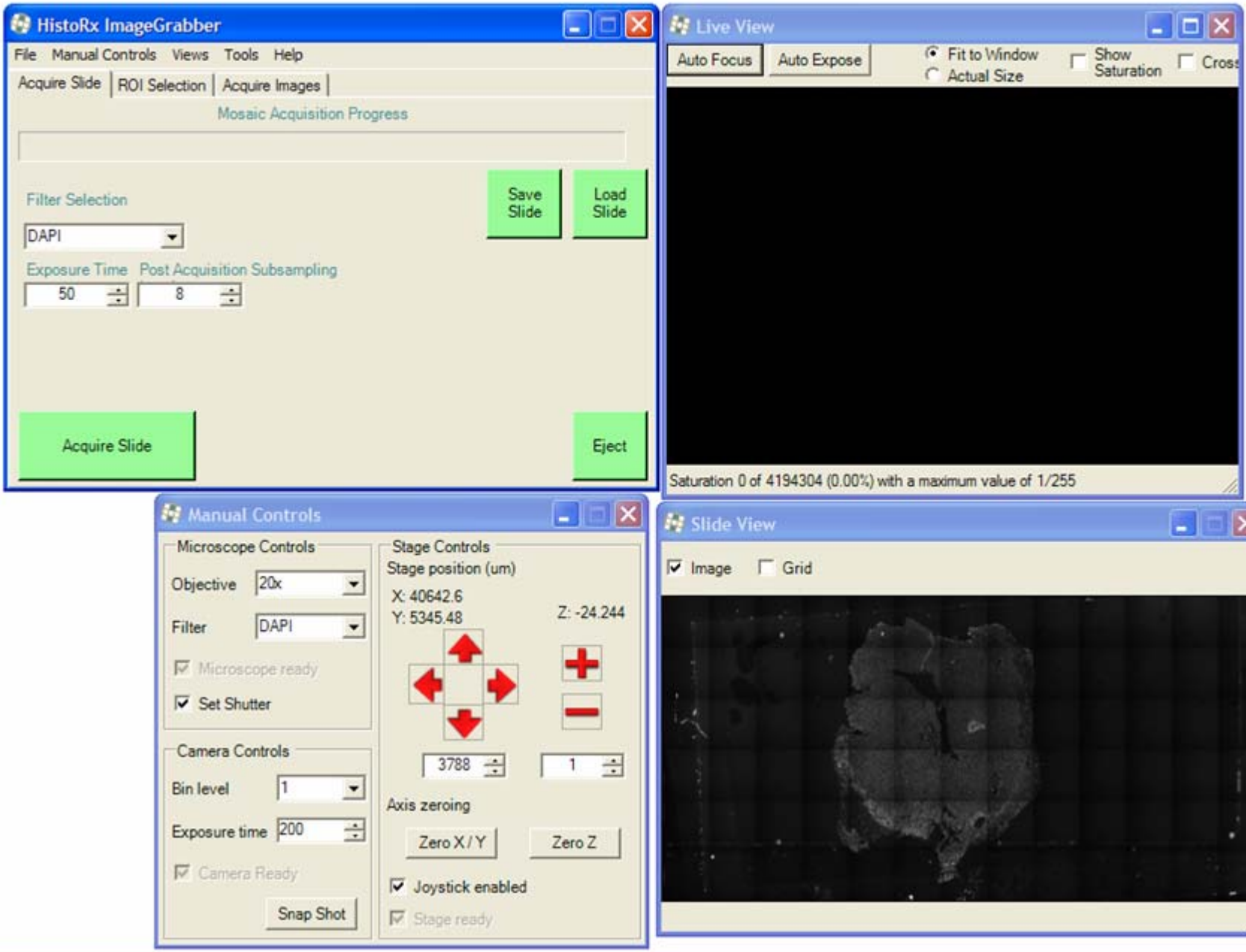

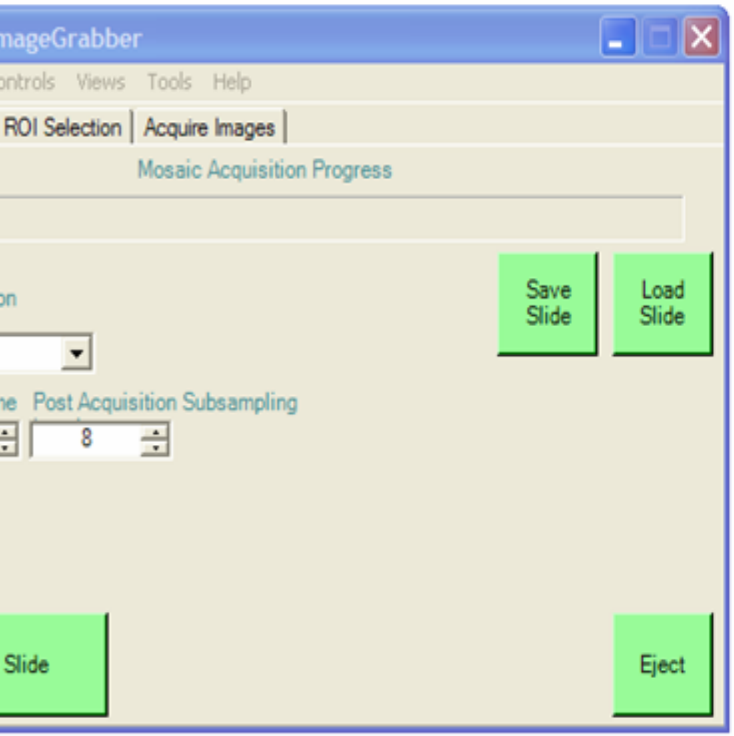
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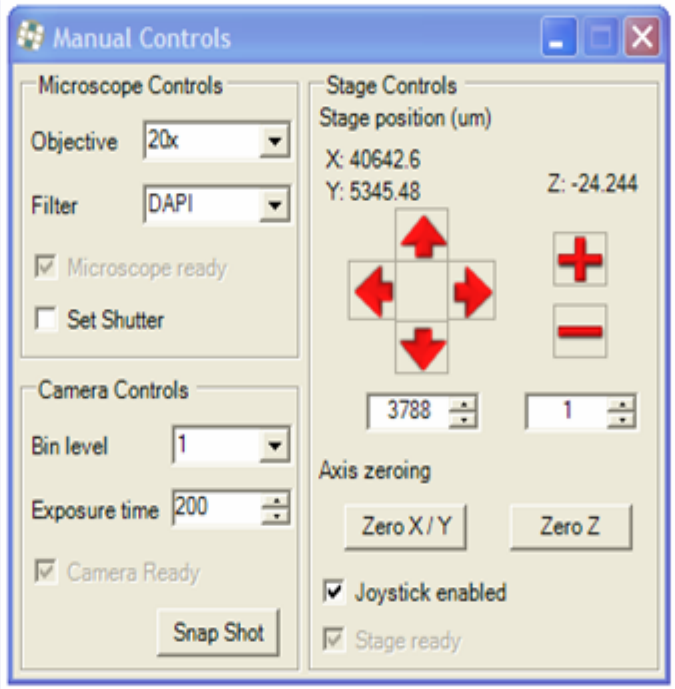
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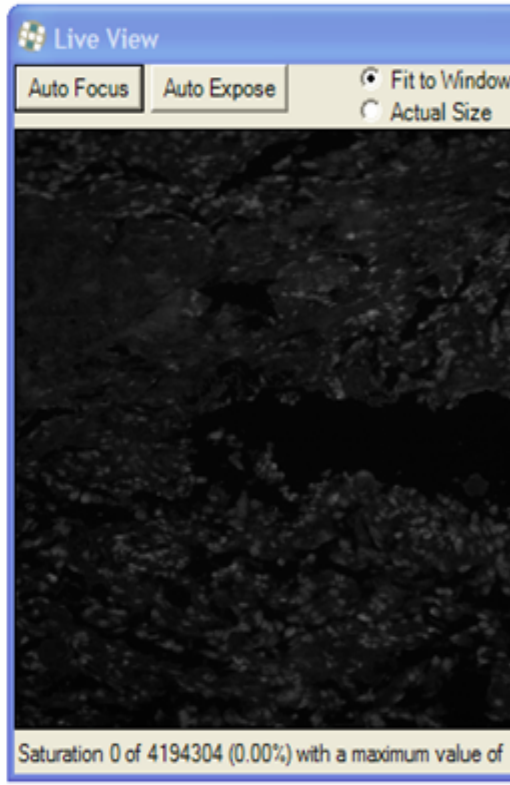
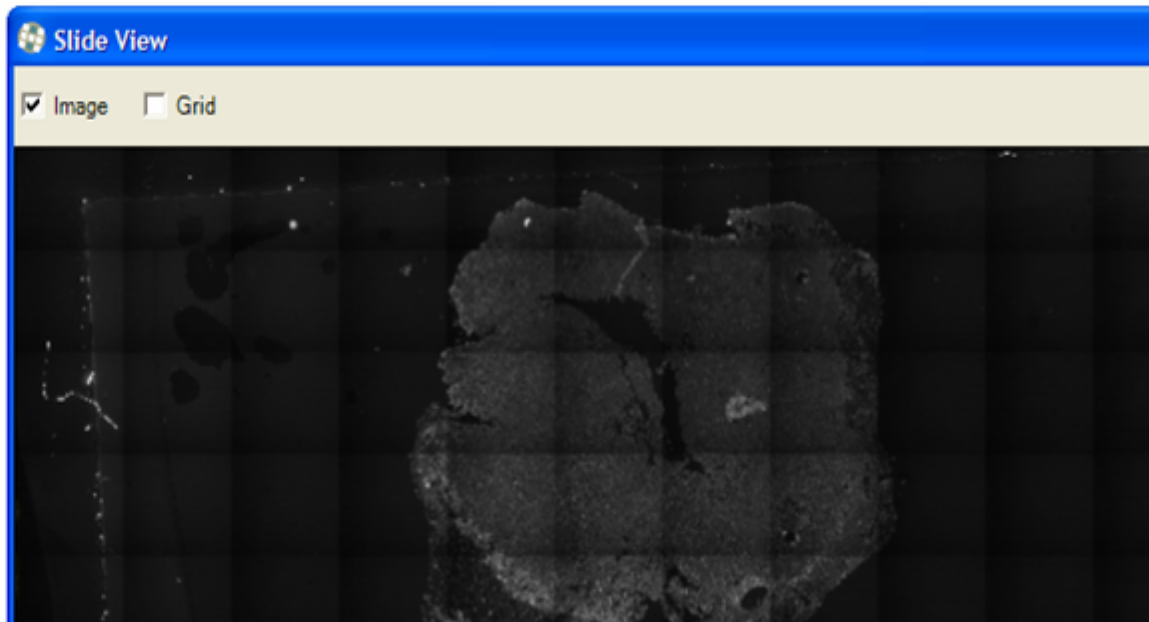
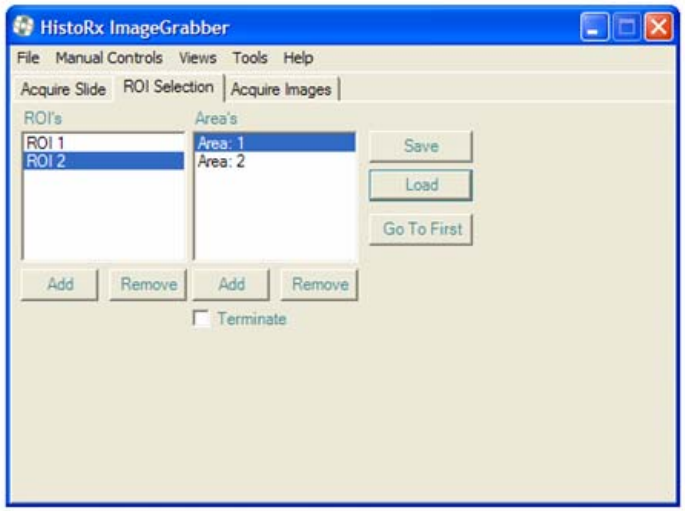
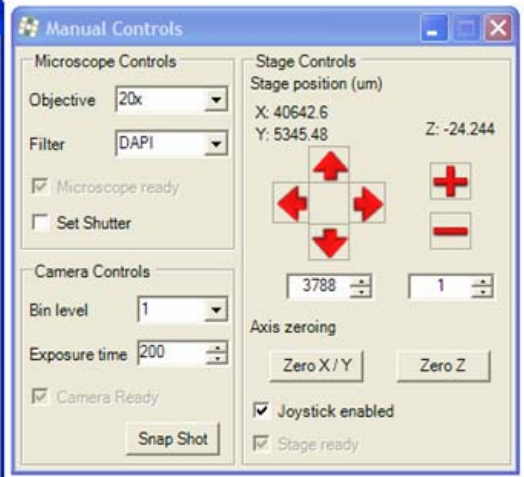
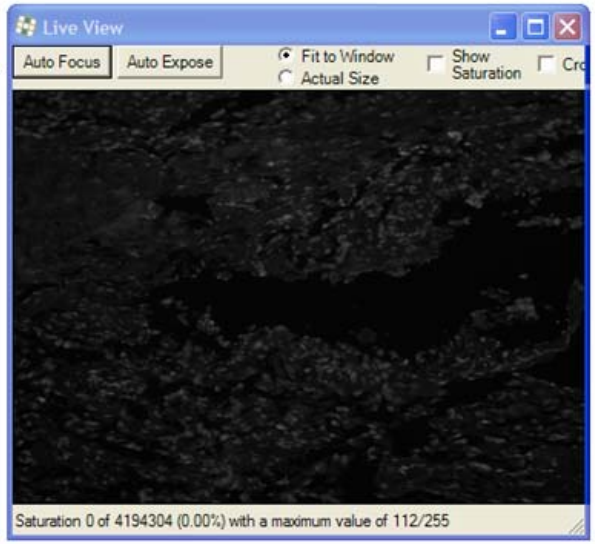
Axis zeroing
Zero X / Y
Zero Z
Joystick enabled
Stage ready

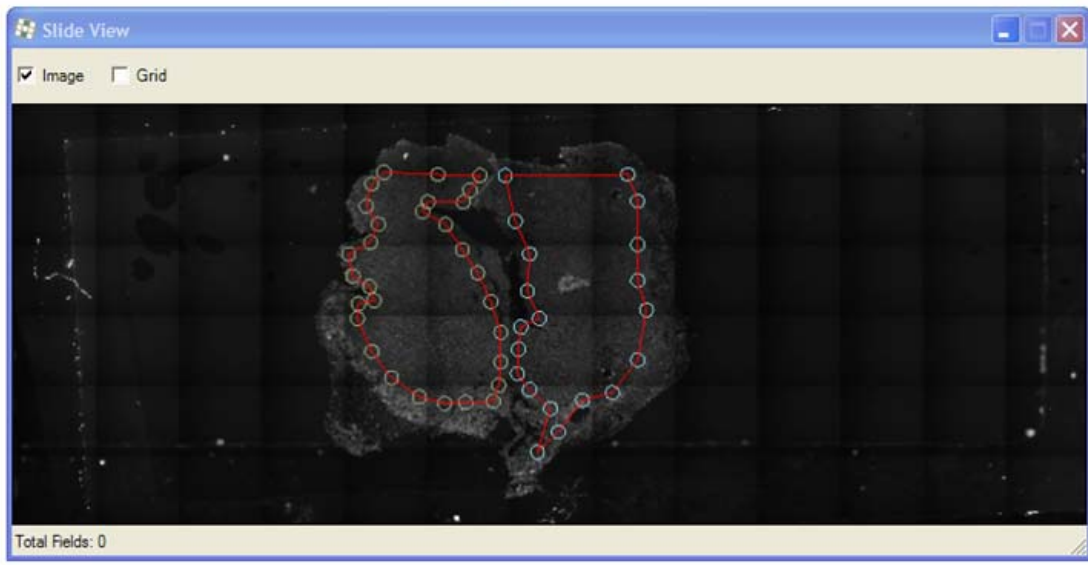

Live View
Auto Focus
Auto Expose
Fit to Window
Actual Size
Saturation 0 of 4194304 (0.00%) with a maximum value of

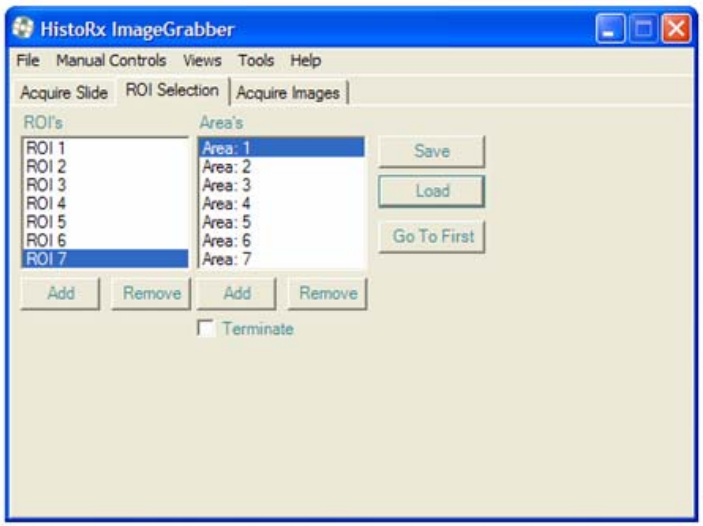

Slide View
Image
Grid

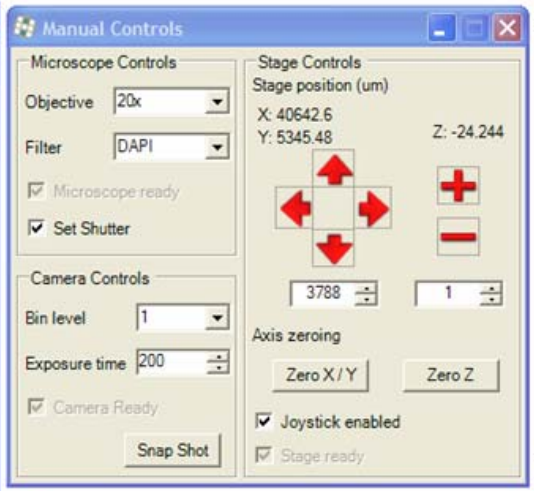

HistoRx ImageGrabber
File
Manual Controls
Views
Tools
Help
Acquire Slide
ROI Selection
Acquire Images
ROI's
Area's
ROI 1
ROI 2
Area: 1
Area: 2
Save
Load
Go To First
Add
Remove
Add
Remove
Terminate

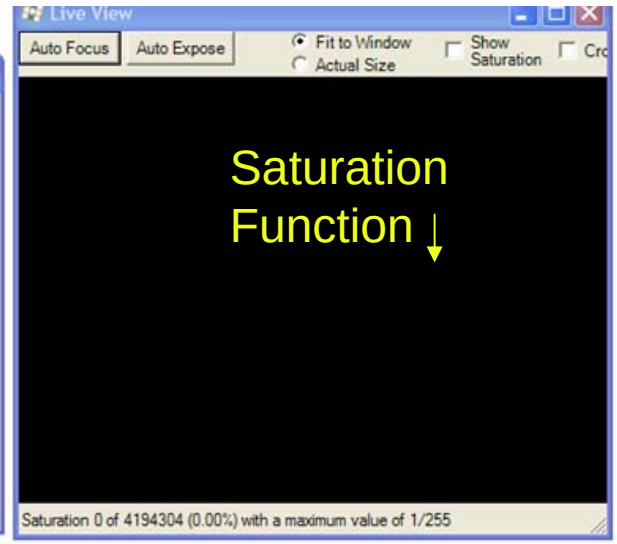

Manual Controls
Microscope Controls
Objective
20x
Filter
DAPI
Microscope ready
Set Shutter
Camera Controls
Bin level
1
Exposure time
200
Camera Ready
Snap Shot
Stage Controls
Stage position (um)
X: 40642.6
Y: 5345.48
Z: -24.244
3788
1
Axis zeroing
Zero X / Y
Zero Z
Joystick enabled
Stage ready


Live View
Auto Focus
Auto Expose
Fit to Window
Actual Size
Show Saturation
Saturation 0 of 4194304 (0.00%) with a maximum value of 112/255


Slide View
Image
Grid
Total Fields: 0


HistoRx ImageGrabber
File
Manual Controls
Views
Tools
Help
Acquire Slide
ROI Selection
Acquire Images
ROI's
Area's
ROI 1
ROI 2
ROI 3
ROI 4
ROI 5
ROI 6
ROI 7
Area: 1
Area: 2
Area: 3
Area: 4
Area: 5
Area: 6
Area: 7
Save
Load
Go To First
Add
Remove
Add
Remove
Terminate


Manual Controls
Microscope Controls
Objective
20x
Filter
DAPI
Microscope ready
Set Shutter
Camera Controls
Bin level
1
Exposure time
200
Camera Ready
Snap Shot
Stage Controls
Stage position (um)
X: 40642.6
Y: 5345.48
Z: -24.244
3788
1
Axis zeroing
Zero X / Y
Zero Z
Joystick enabled
Stage ready


Live View
Auto Focus
Auto Expose
Fit to Window
Actual Size
Show Saturation
Saturation Function ↓
Saturation 0 of 4194304 (0.00%) with a maximum value of 1/255

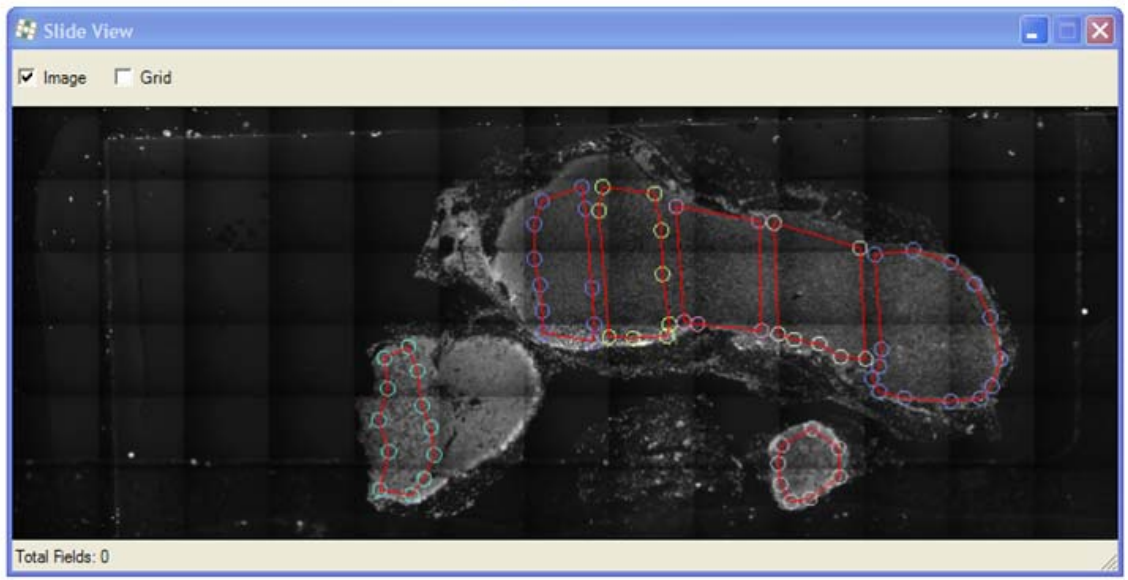
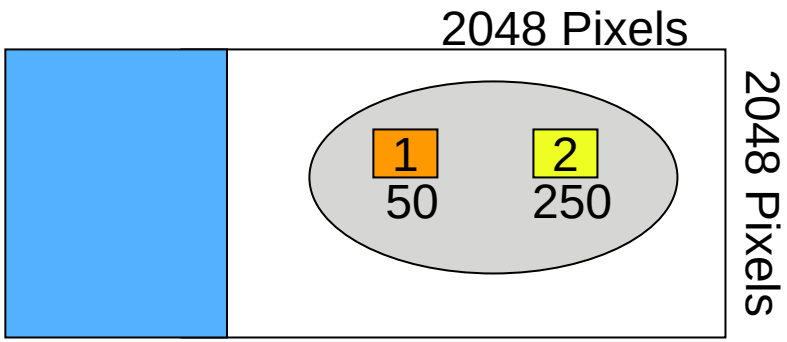

Slide View
Image
Grid
Total Fields: 0

Image Grabber: AQUA Score Normalization through Saturation Function

[1-256: shade of gray/degree saturation]

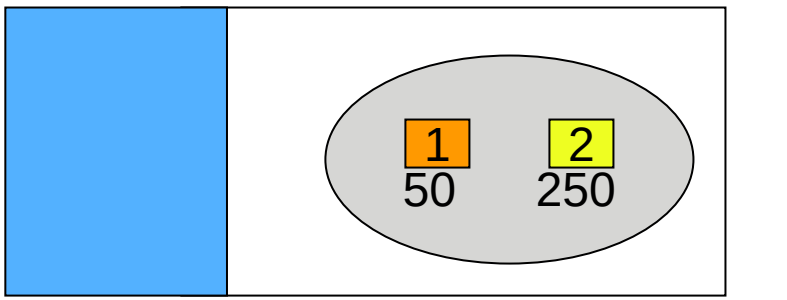
$P_i = \frac{P(\text{saturation})}{\text{Exp time}}$

$\text{Power} = \frac{\text{Intensity}}{\text{Time}} = P_i$



Slide 1: Cy5 = **50ms**

$Pi = \frac{250}{50ms} = \mathbf{5 = P}$



Slide 2: Cy5 = **250ms**

$Pi = \frac{250}{250ms} = \mathbf{1 = P}$

Takes 5X Longer to reach 250; P same for both

- Each pixel: 1-256
- Calculate avg total pixel saturation
- Black --> White
- Unsaturated --> Saturated

P_{SAT}/ 4,194,304 Total Pixels= x 100 = % saturation

Image Capture: Data Set Descriptors

- Project Parameters
  - 184.4 GB total
  - 149 folders (1 folder/case)
  - 845 files

Typical Case Slide Size Range:

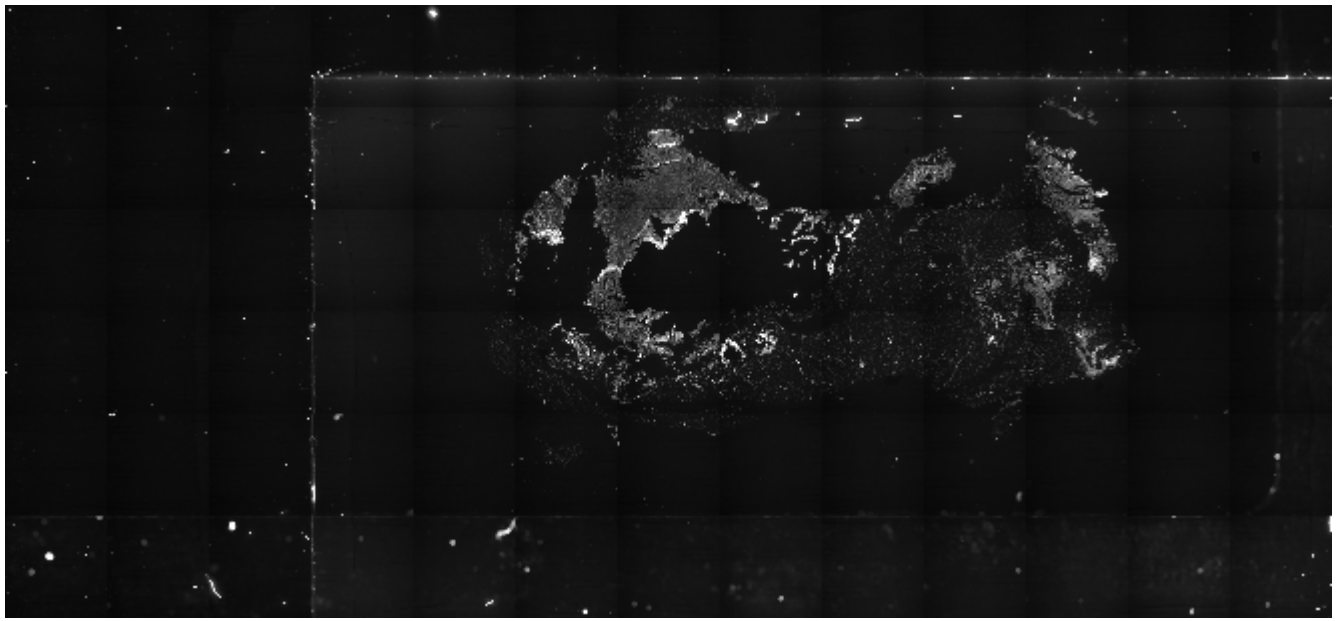
Low: Case # H77
90 MB
1 ROI/TMA
10 images total

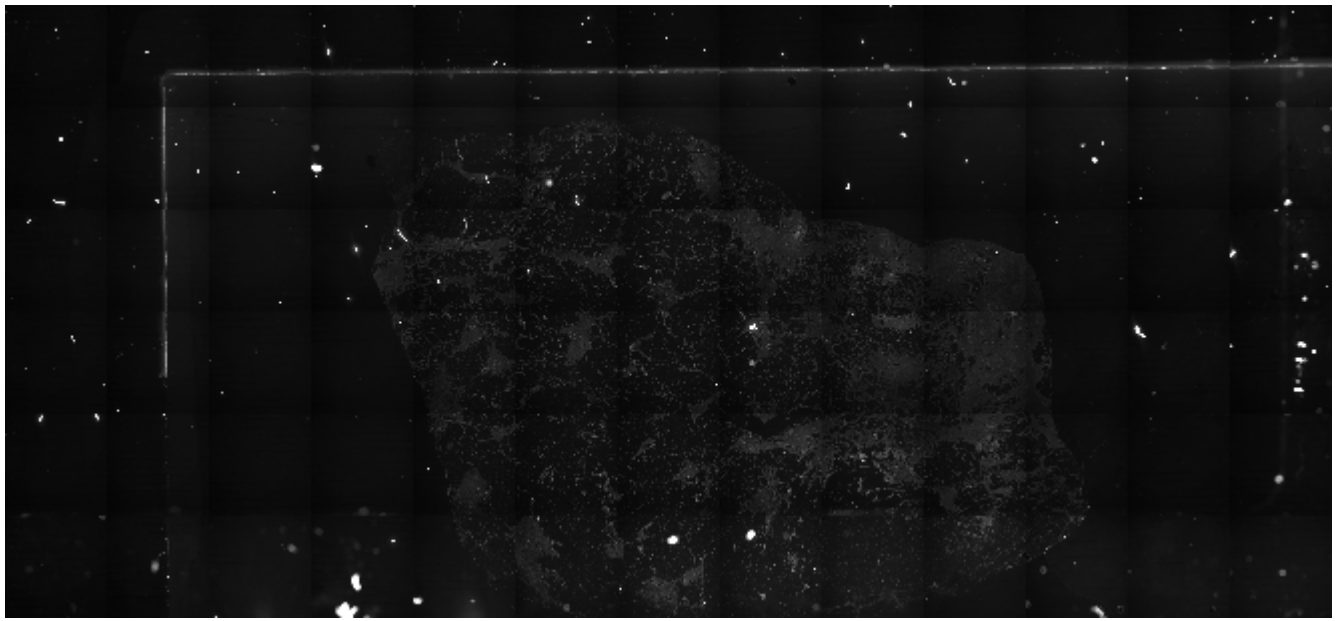
High: Case # H99
6.94 GB
8 ROI/TMAs
519 images total

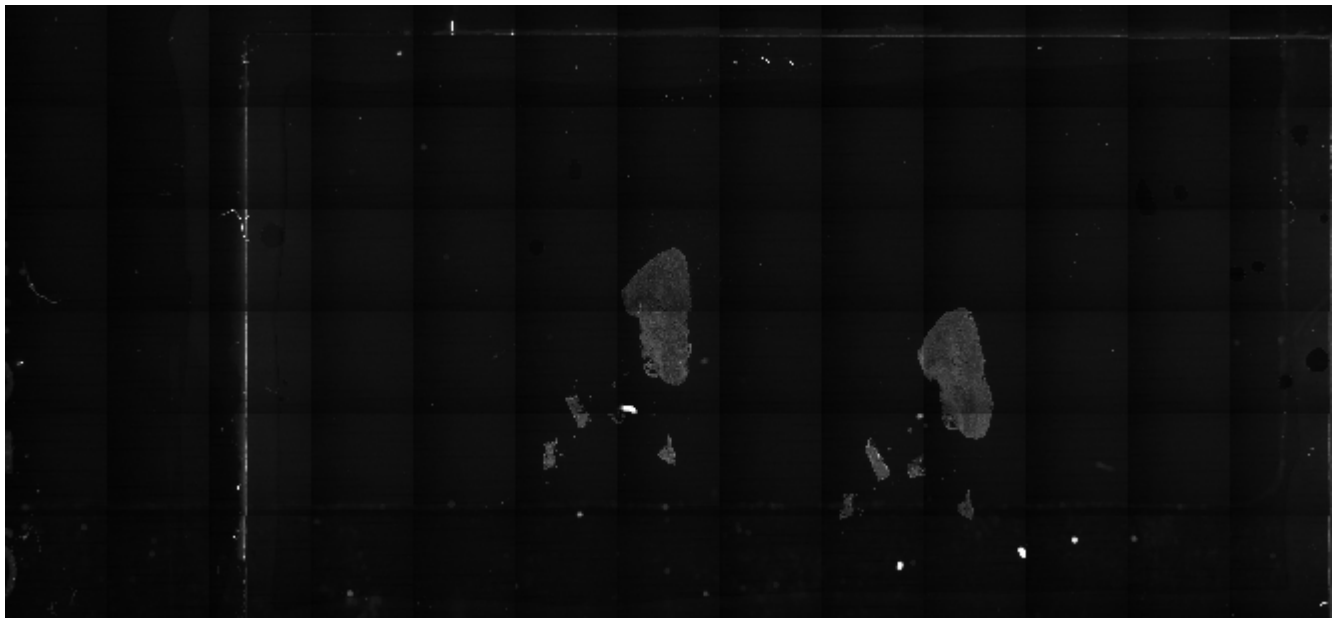
Image Capture Challenges

- Only 61% of H&Es available
- Tissue distortion after staining: folding, tearing, shearing, erosion
- Sections not planar; Multiple ROIs
- First use 20x to “explore” tissue sections and plane of focus, then set ROIs Collapse any ROI into 2 if still out of focus
- PM3 makes many, many files! Label appropriately for patient cross-matching late
- Criteria for Excluding Cases (determined at beginning of study):
  1. No tissue on specimen slide
  2. Heavy tissue erosion: >95% of tissue gone
  3. Validation: out of focus, artifacts (uncropable)

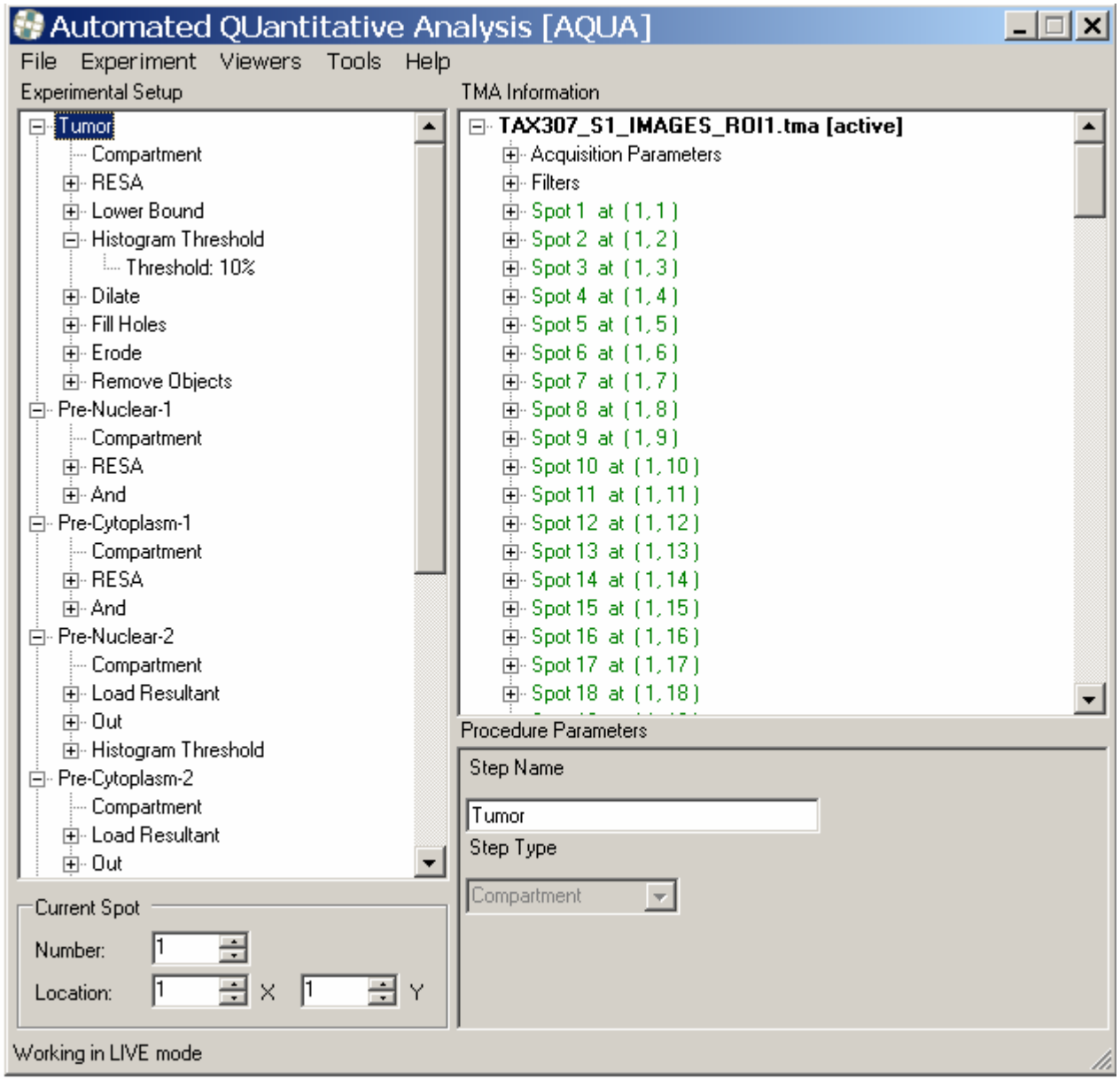
Total Cases Excluded during image capture: 16
(16/140= 11.4% Excluded, 88.6% Retained)







Quantitative analysis of specimens: HistoRx AQUA



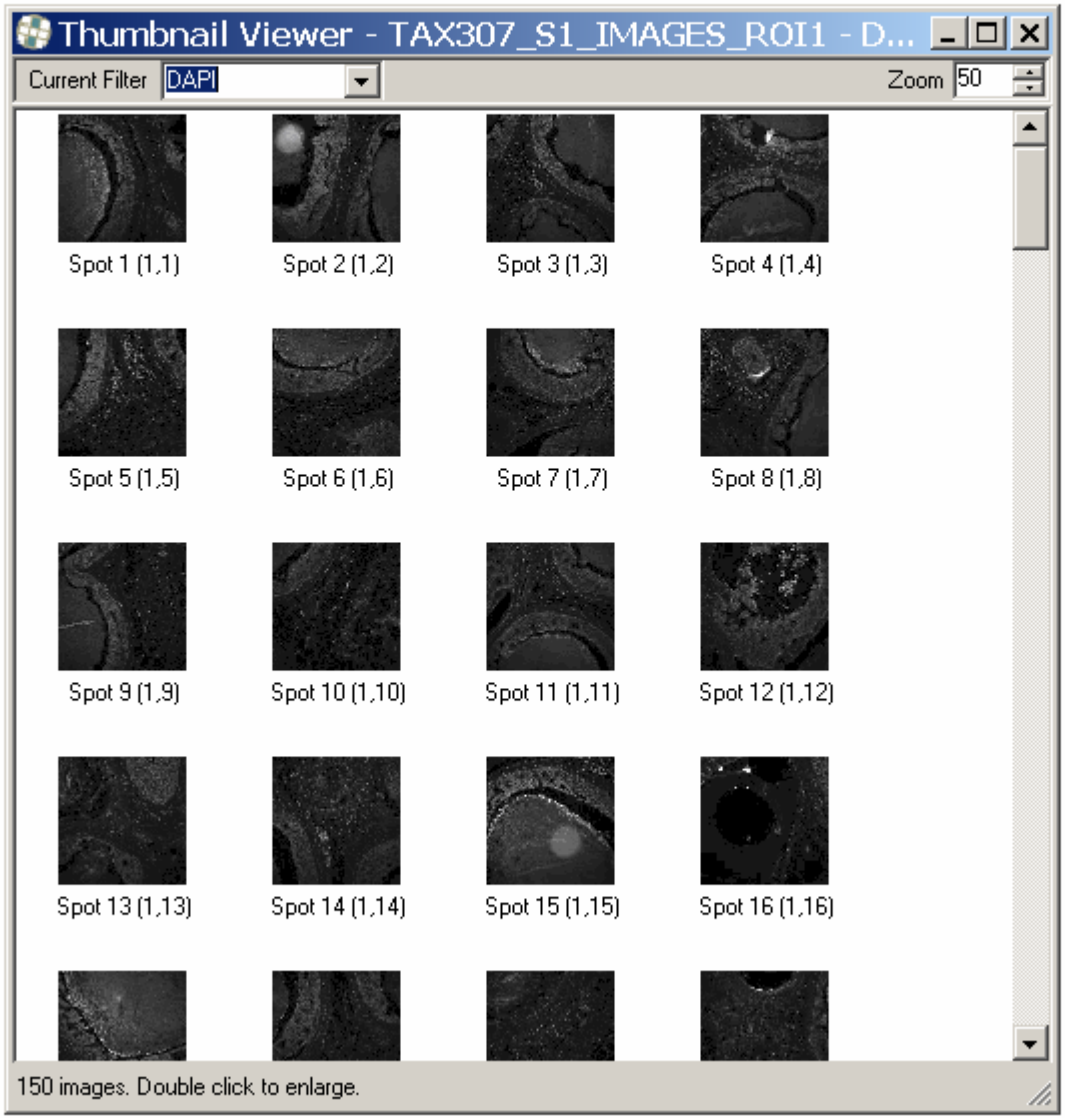
Quantitative analysis of specimens: HistoRx AQUA

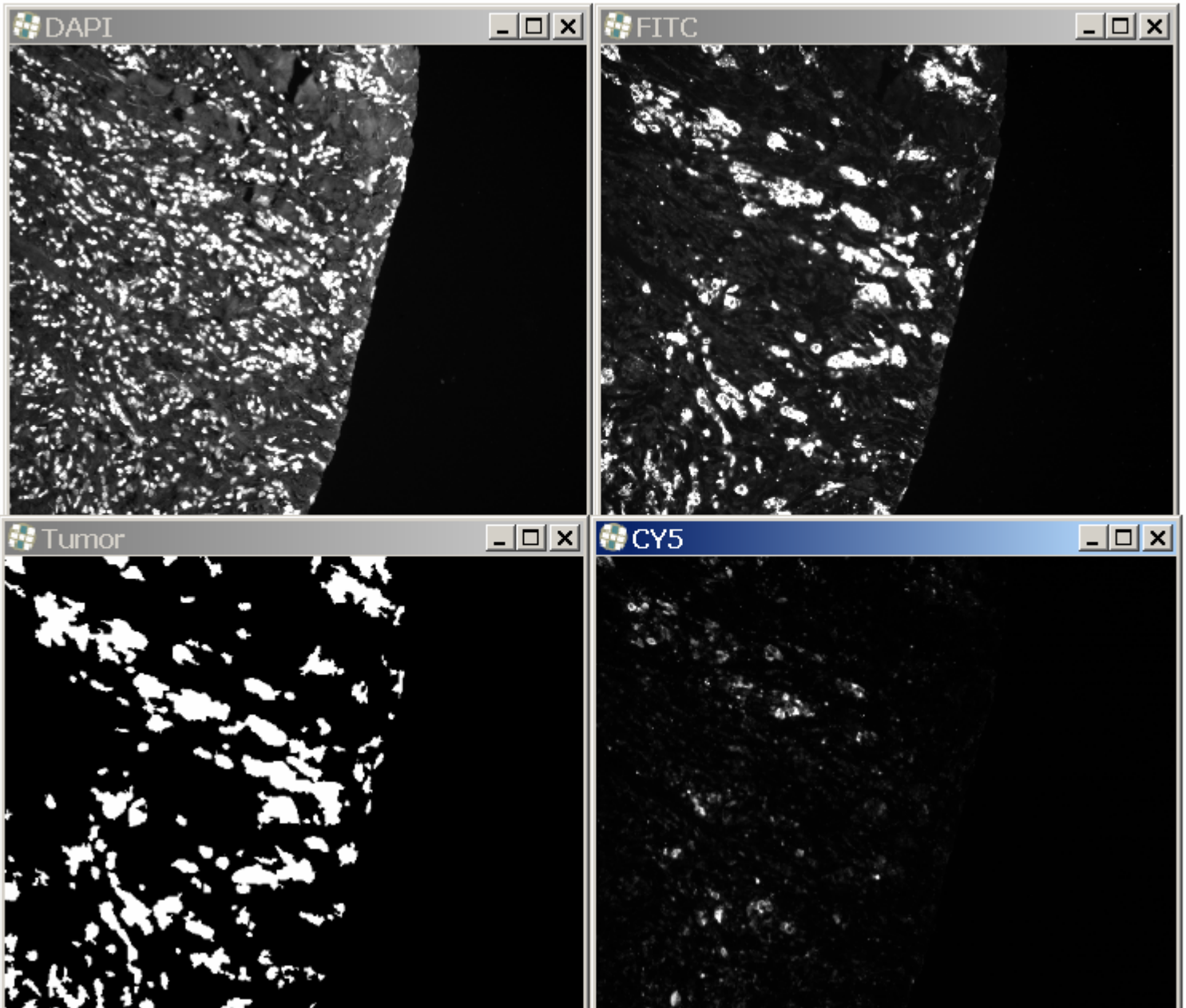
Strengths:

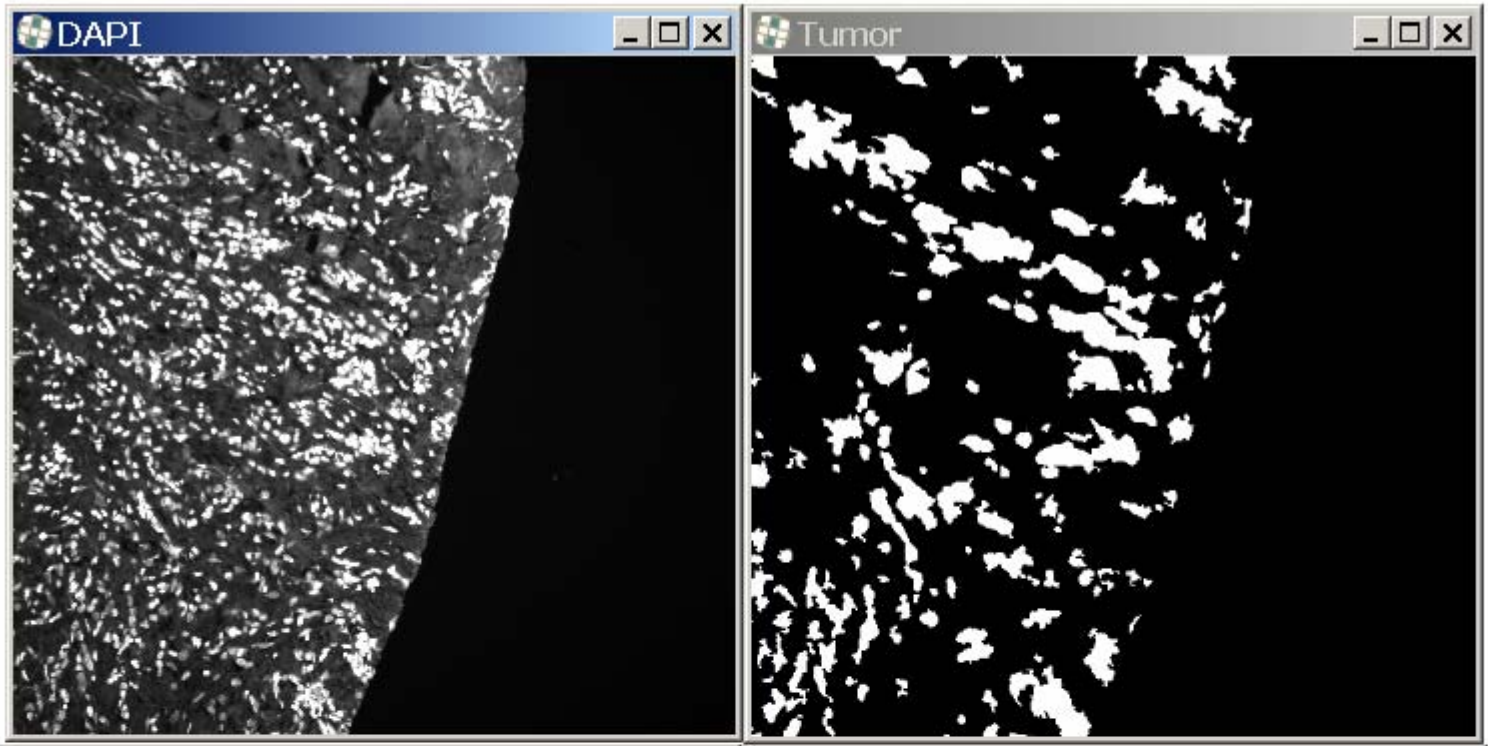
- Rapid experiment run-time: ~11 TMA images per minute
- Tumor Histogram Threshold: 10-20% and can be adjusted per slide
- Relatively small template and analysis file size: <100 MB
- User-friendly interface with multiple ways to examine images

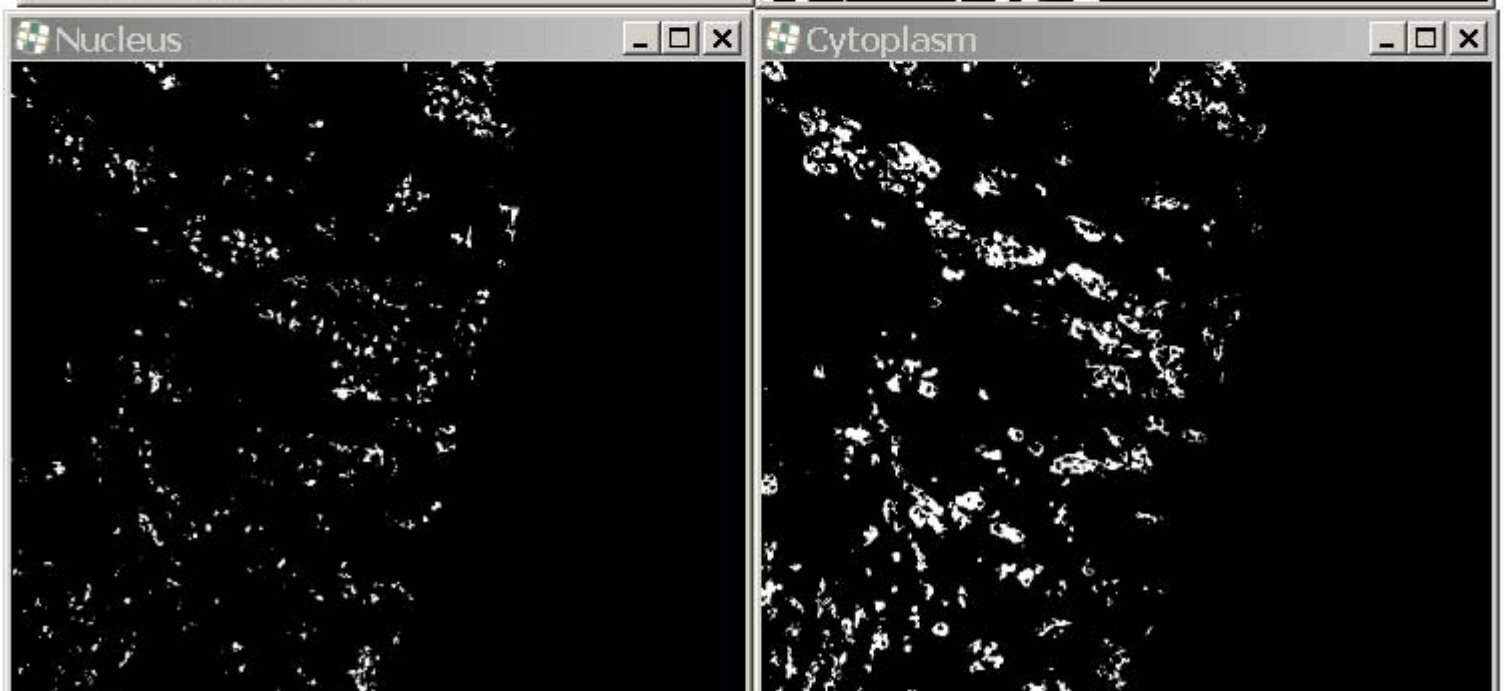
Limitations:

- No Cropping! so entire image must be discarded
- Still some bugs so error messages are common with too many open windows
- Exposure times may not register properly in manual mode

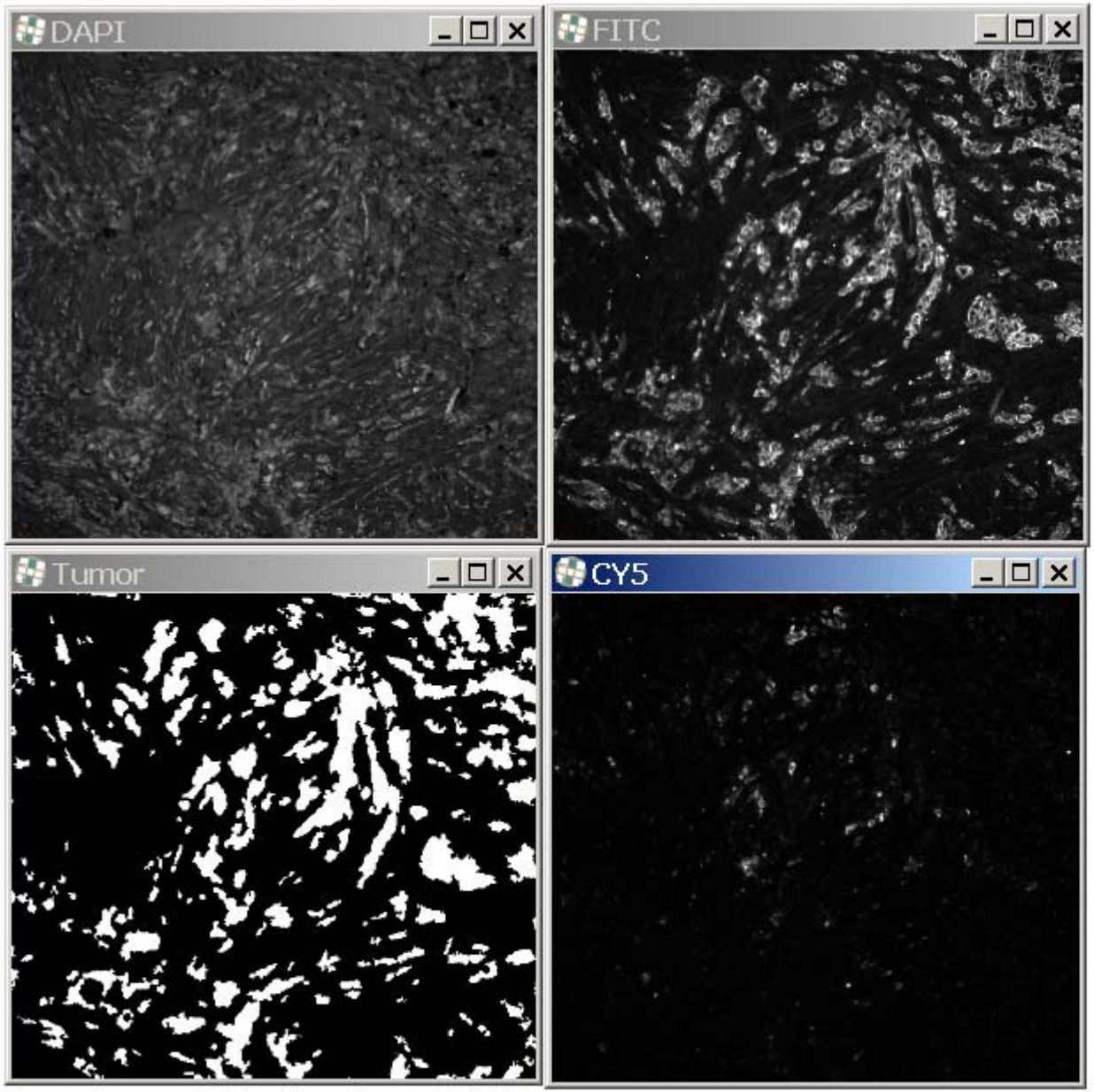

Thumbnail Viewer - TAX307_S1_IMAGES_ROI1 - D...
Current Filter
DAPI
Zoom
50
Spot 1 (1,1)
Spot 2 (1,2)
Spot 3 (1,3)
Spot 4 (1,4)
Spot 5 (1,5)
Spot 6 (1,6)
Spot 7 (1,7)
Spot 8 (1,8)
Spot 9 (1,9)
Spot 10 (1,10)
Spot 11 (1,11)
Spot 12 (1,12)
Spot 13 (1,13)
Spot 14 (1,14)
Spot 15 (1,15)
Spot 16 (1,16)
150 images. Double click to enlarge.


DAPI
FITC
Tumor
CY5

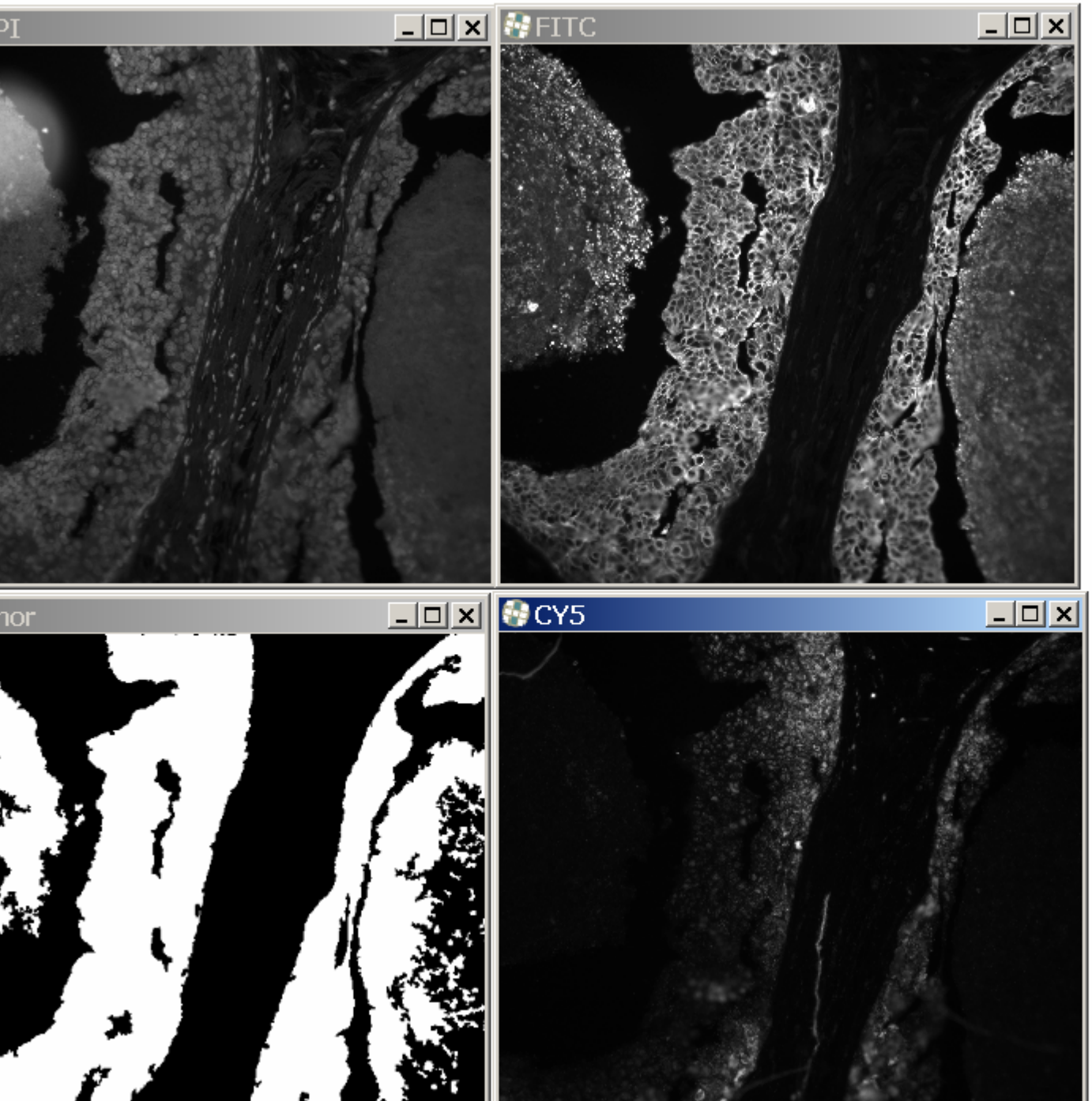




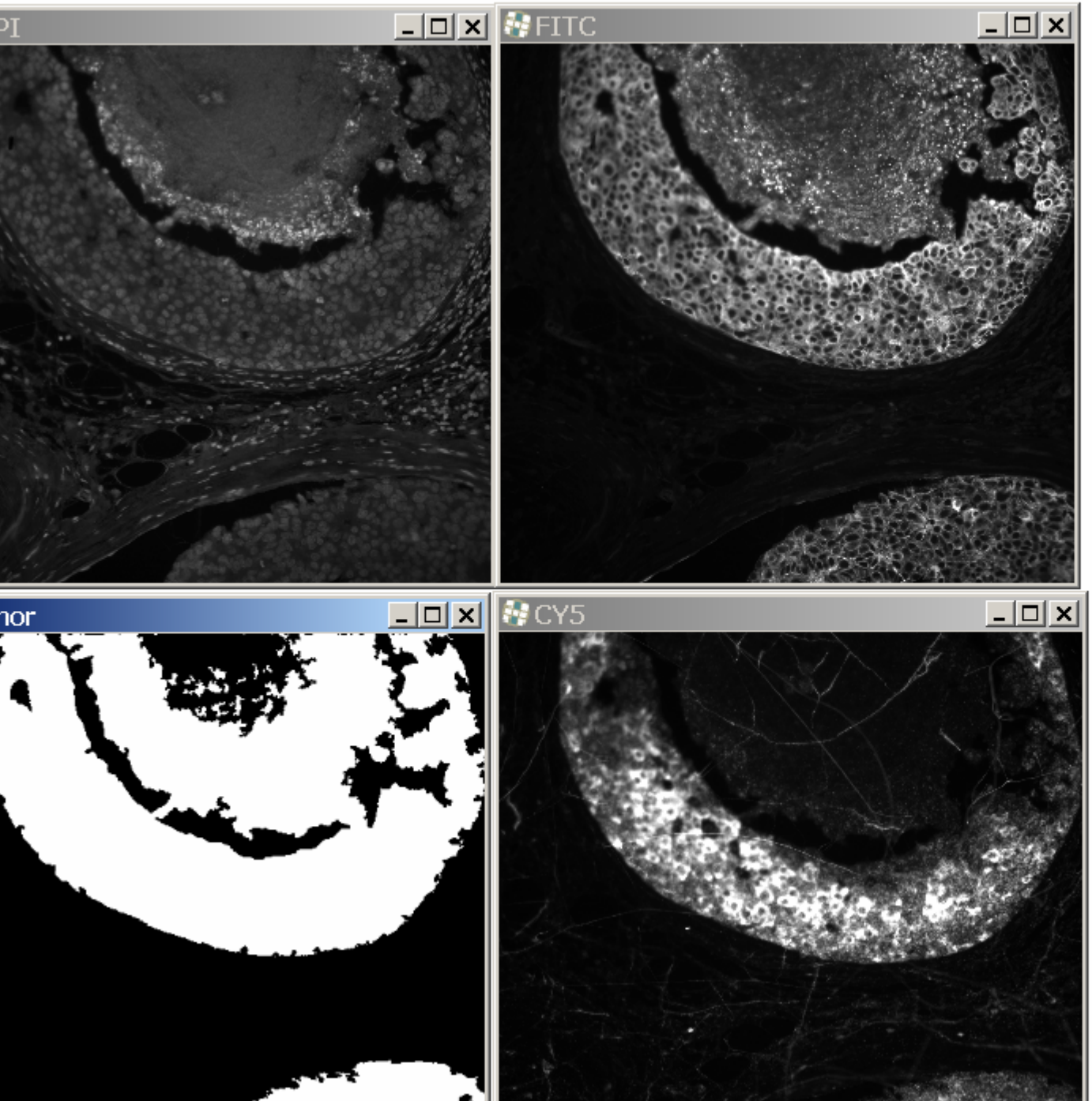
H2
Image 7



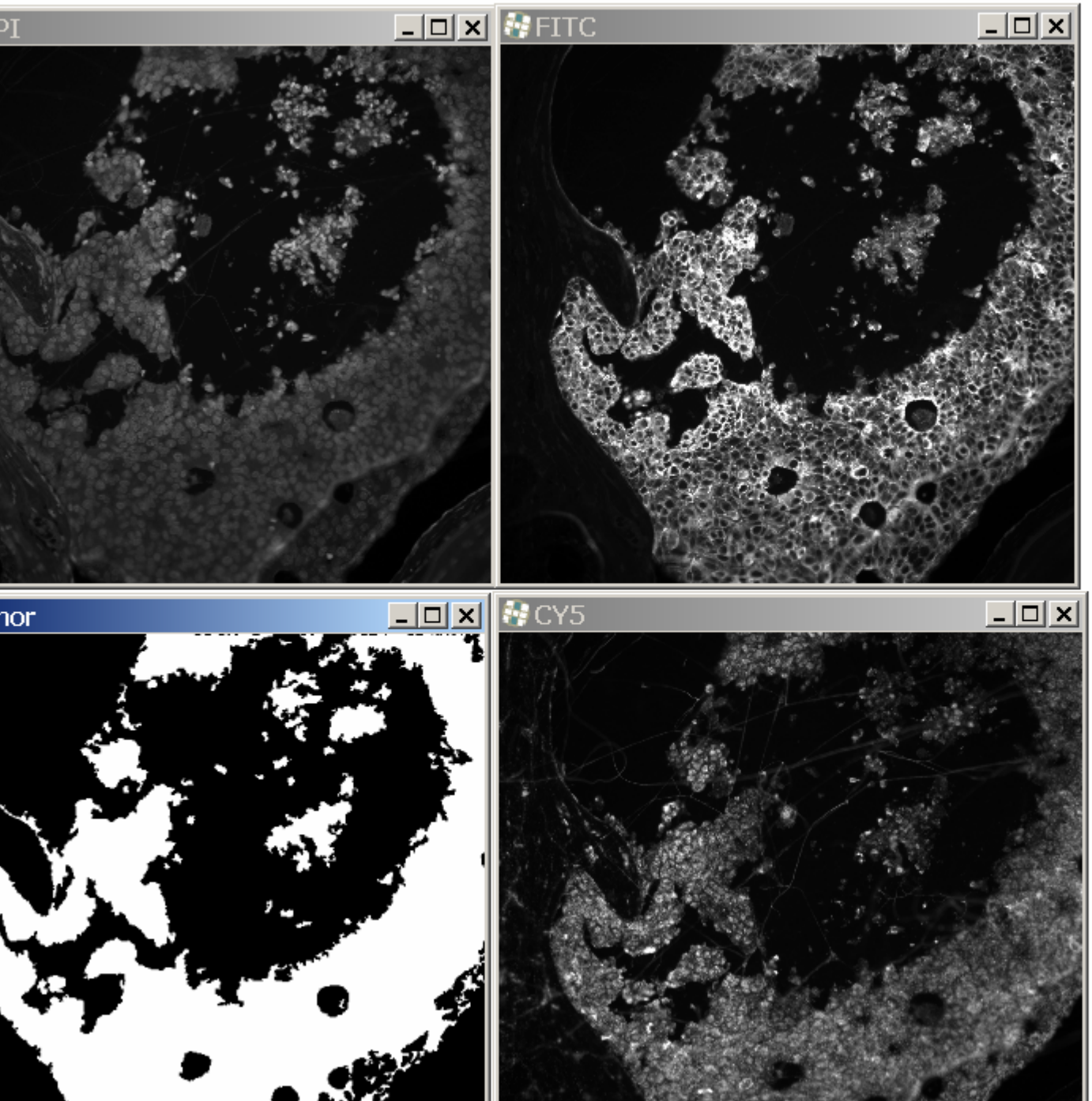
H2
Image 15



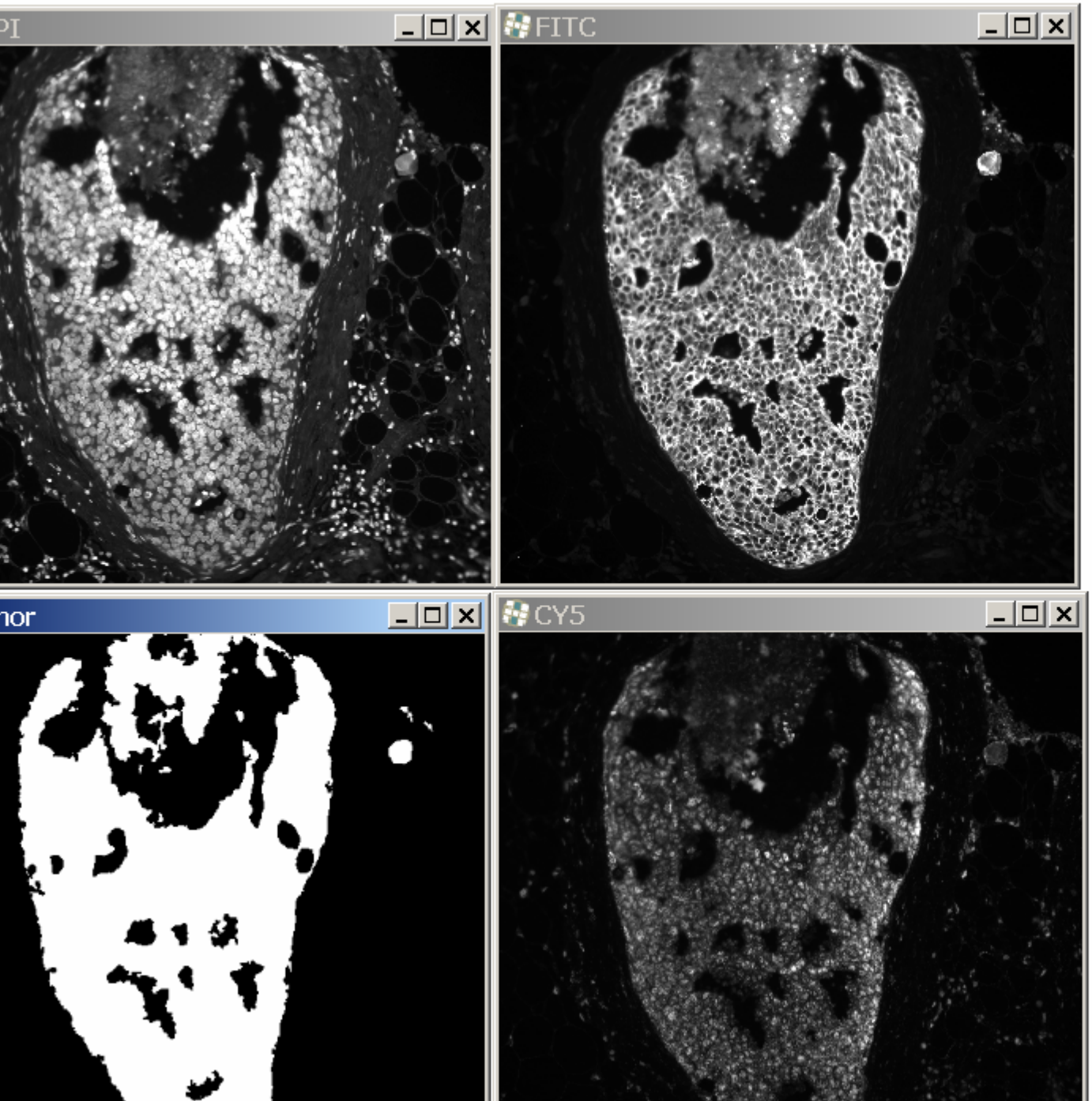
H1
Image 2



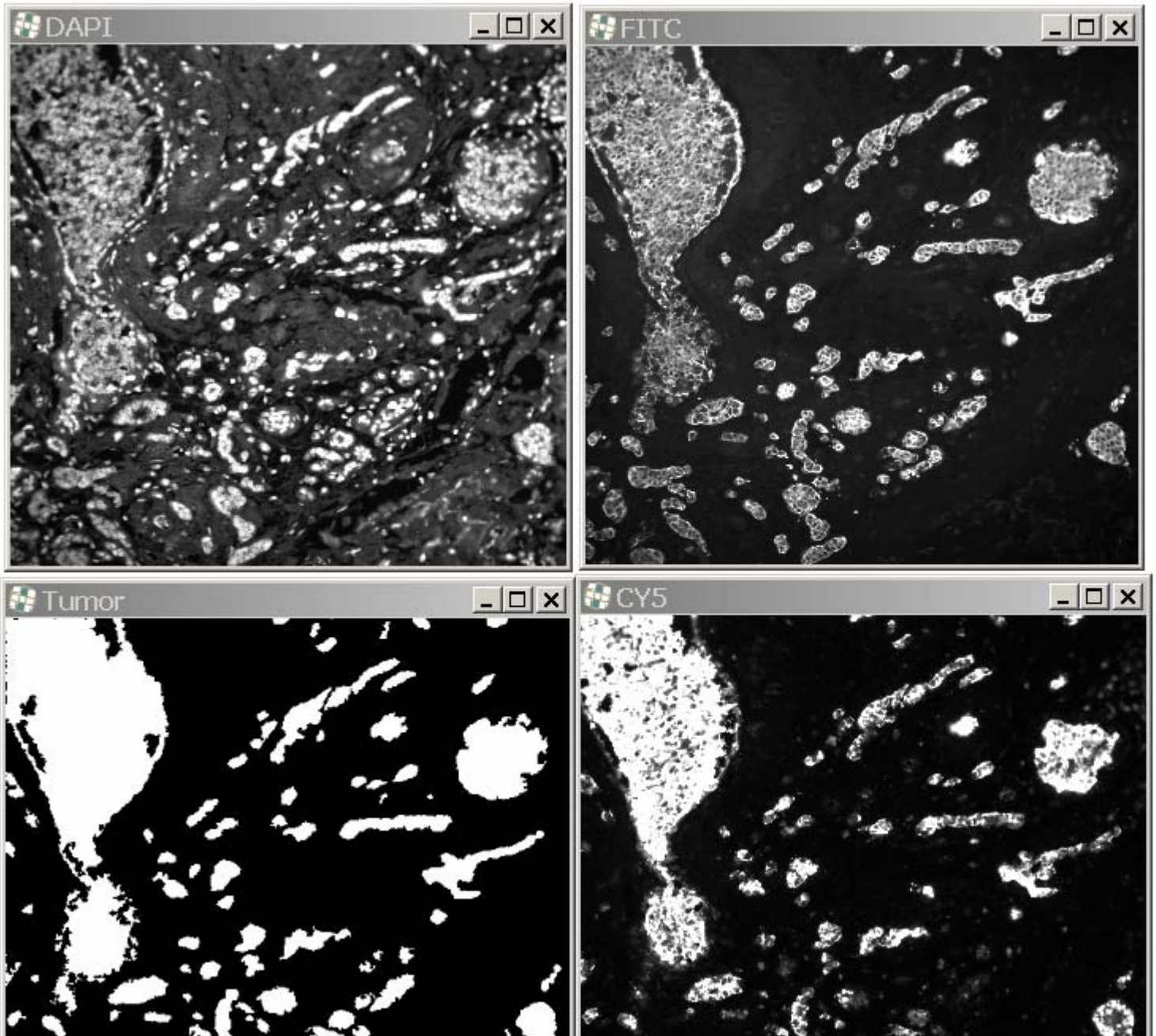
H1



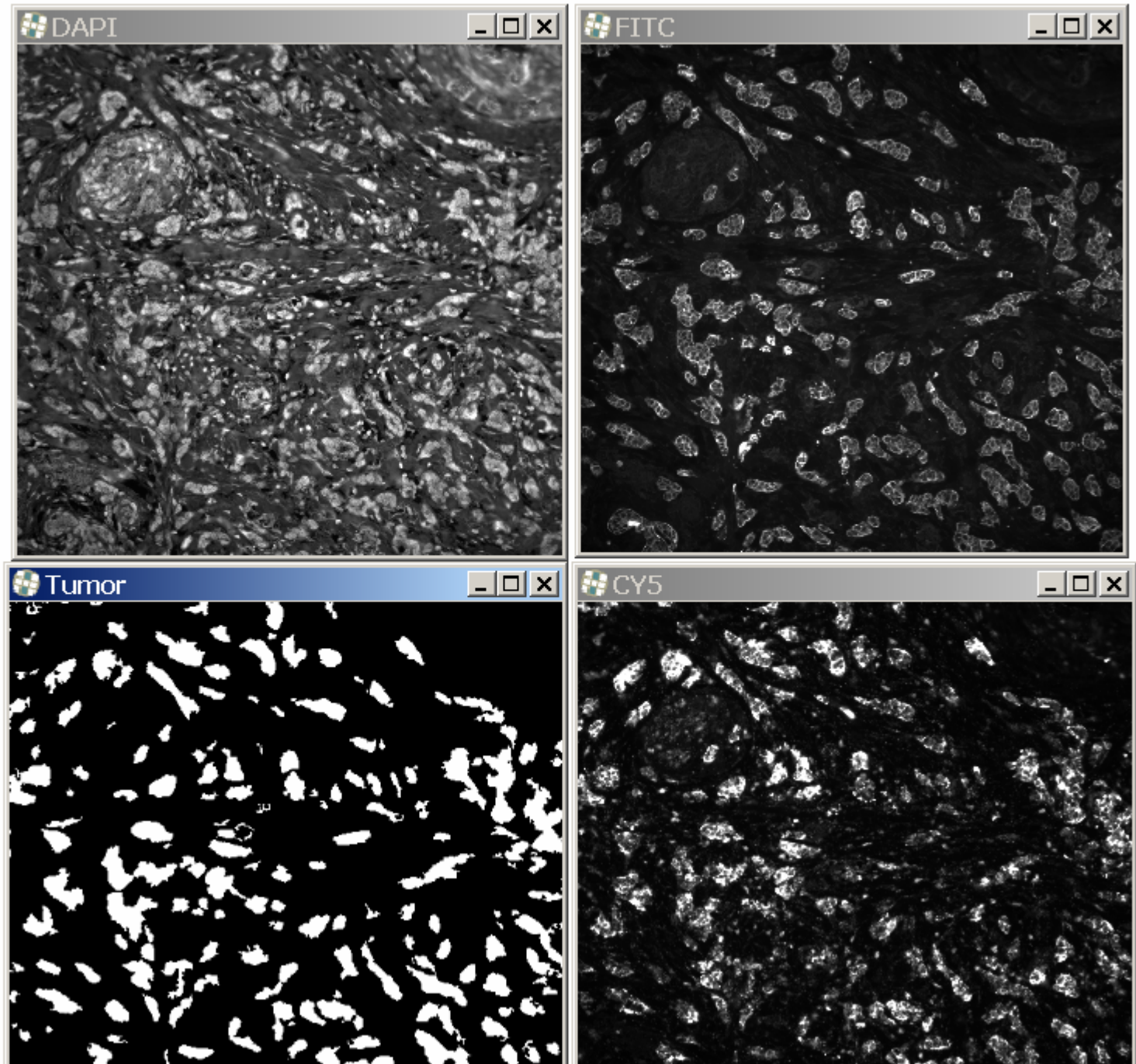
H1



H1



H92



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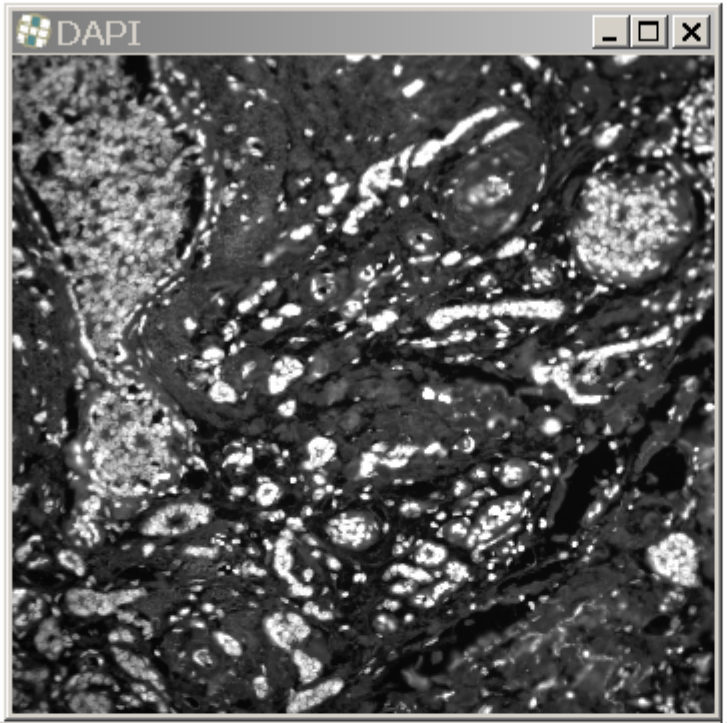
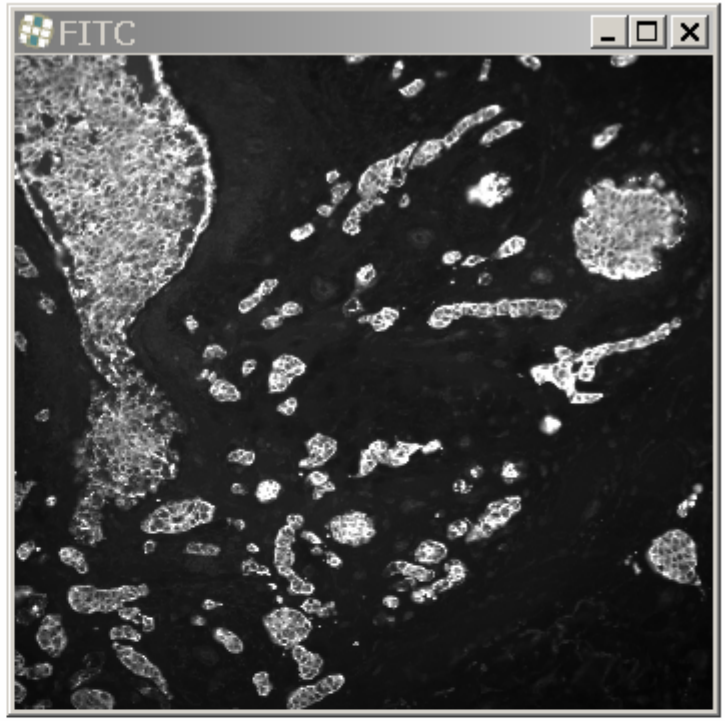
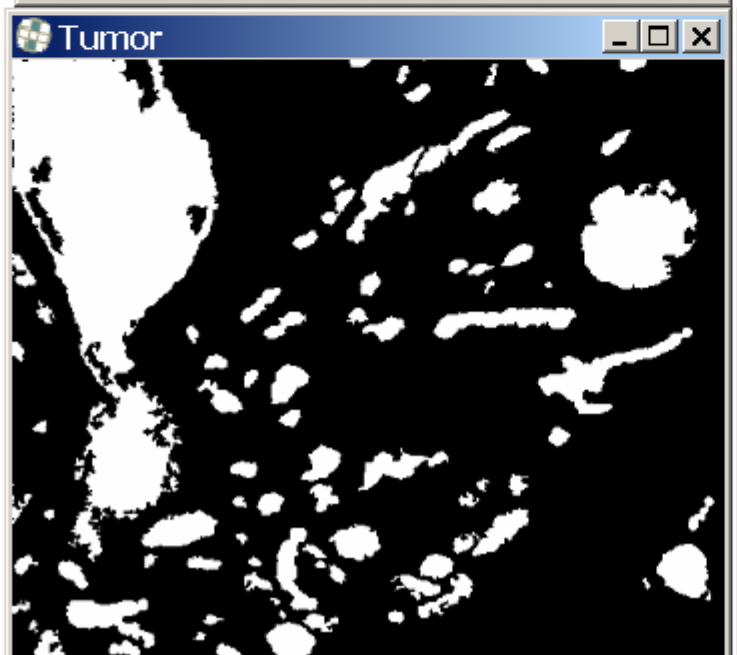
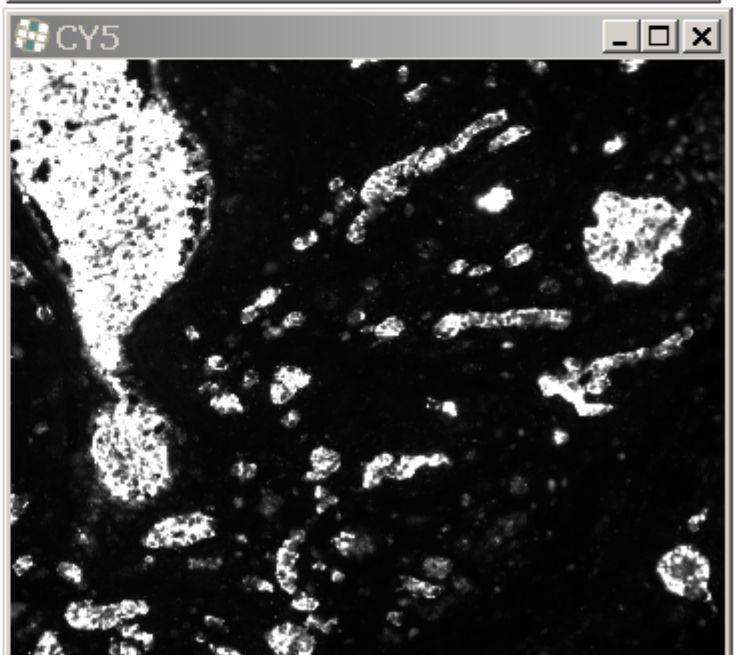
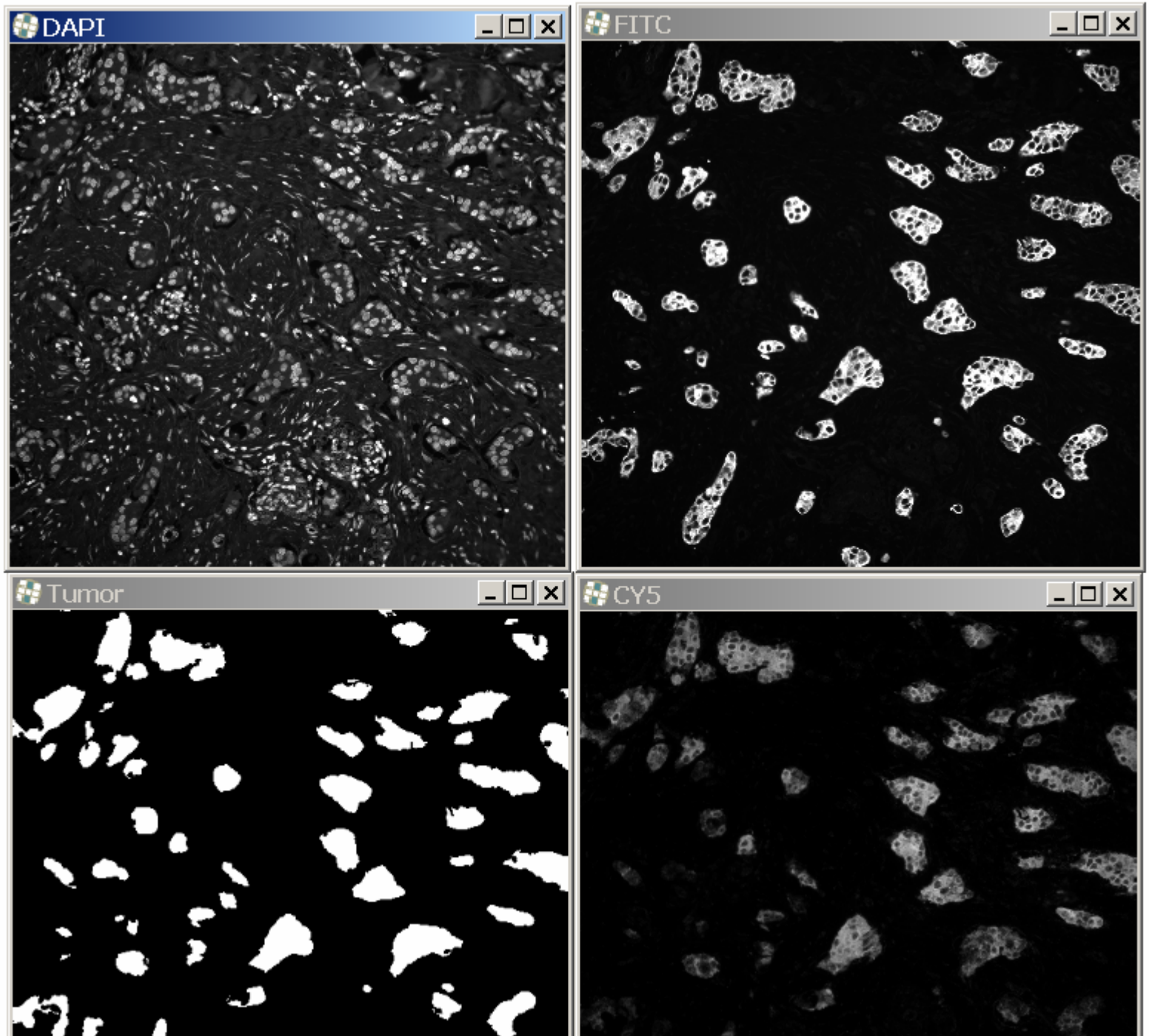


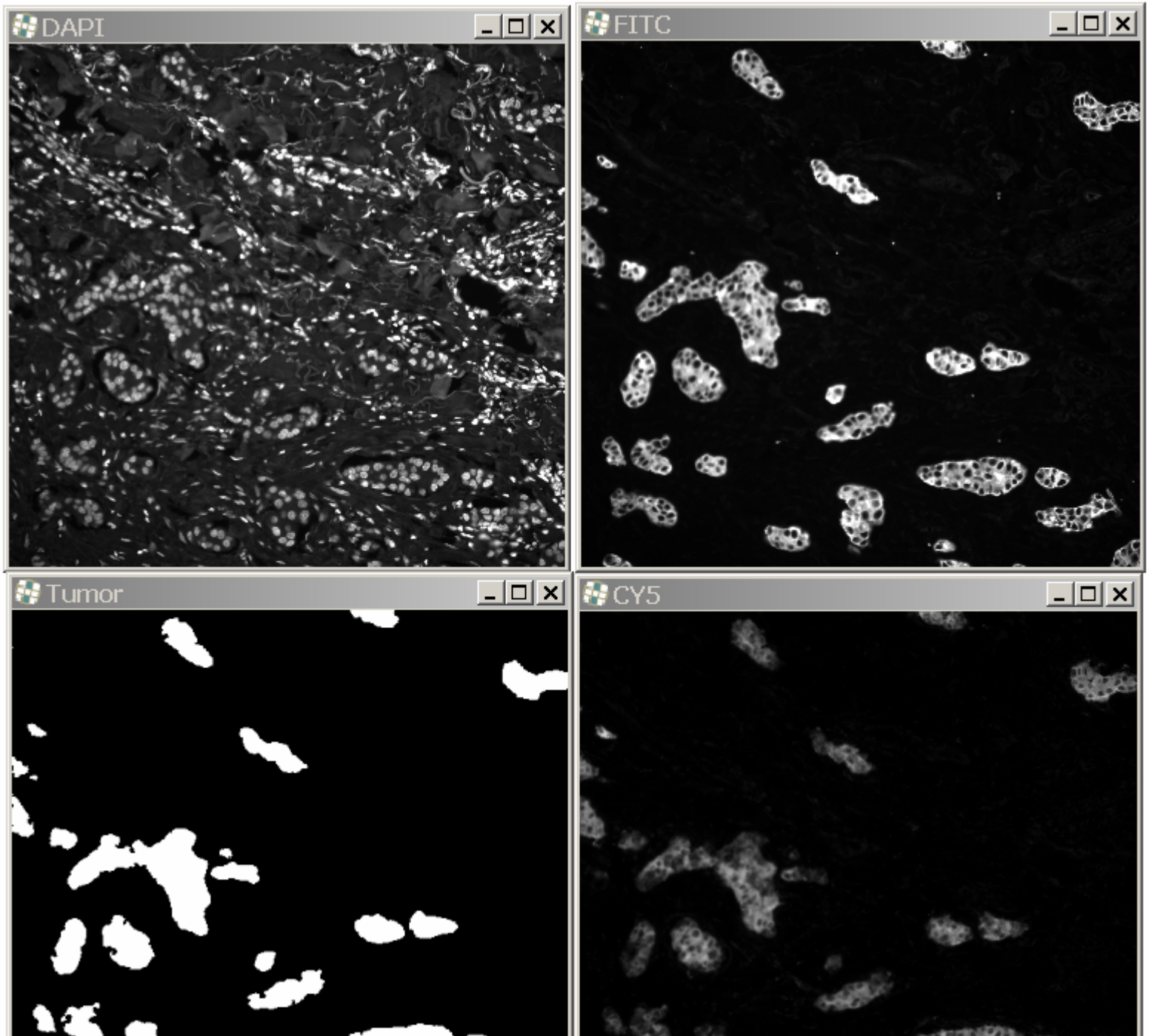


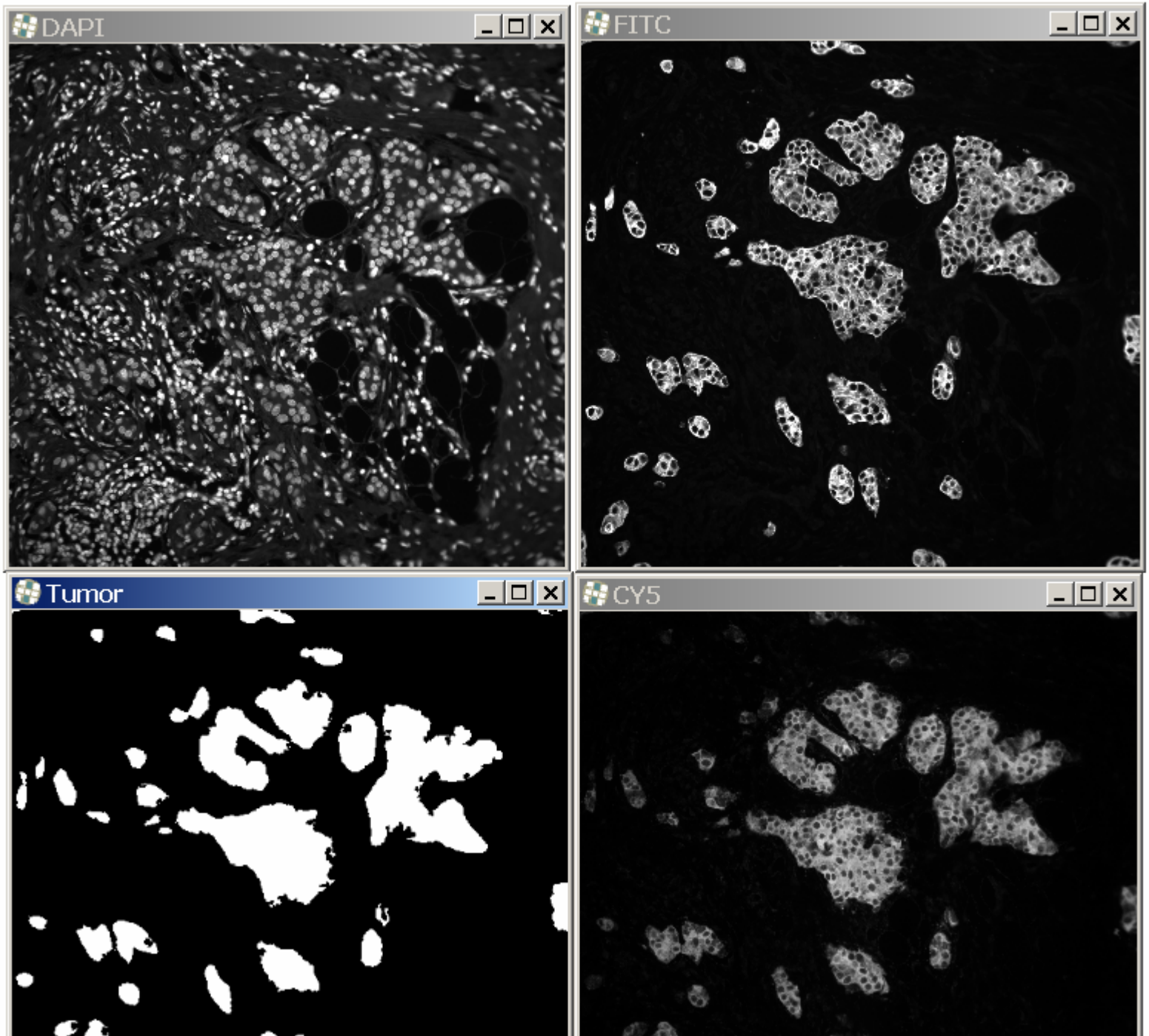
H92
Imag

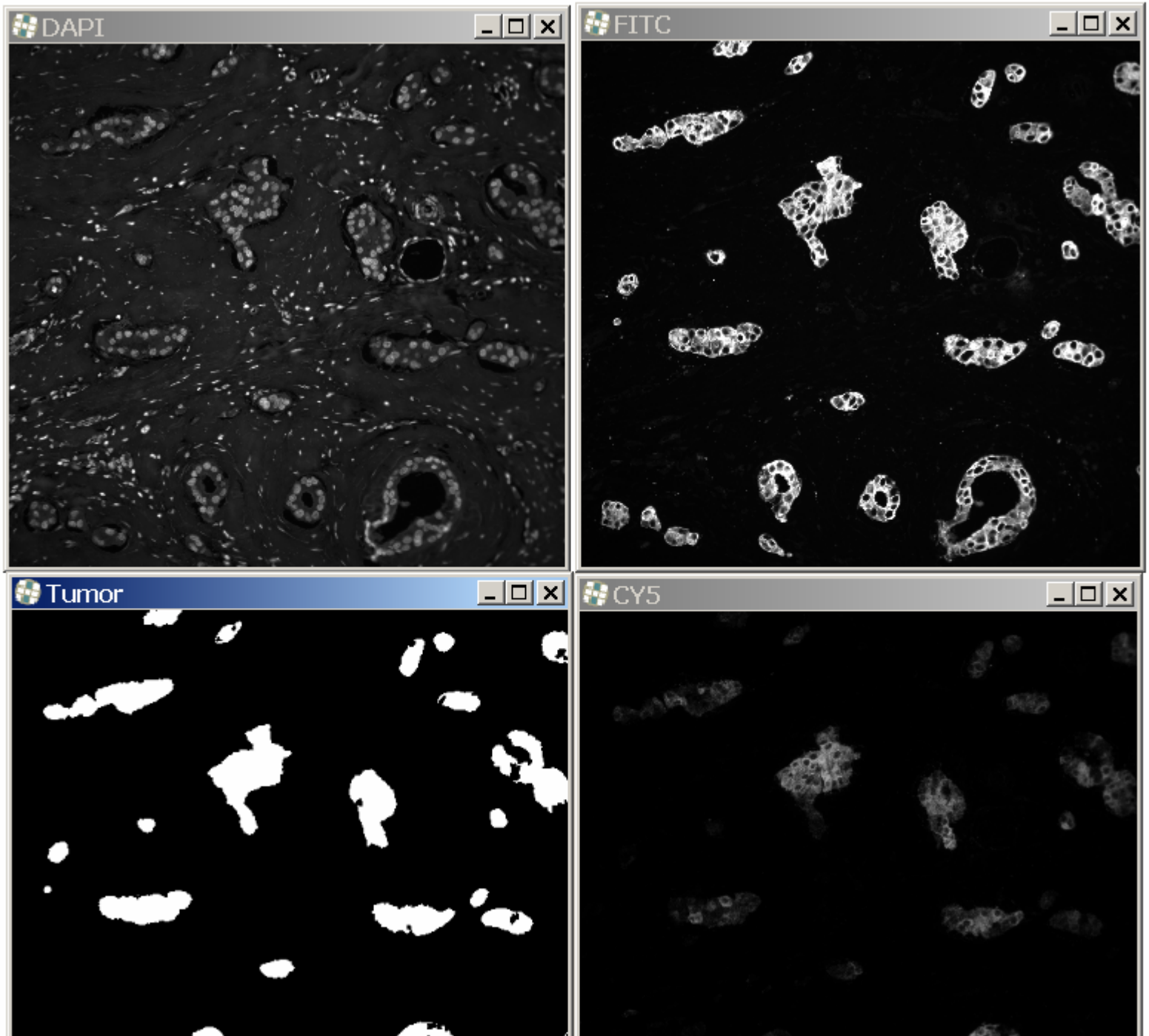


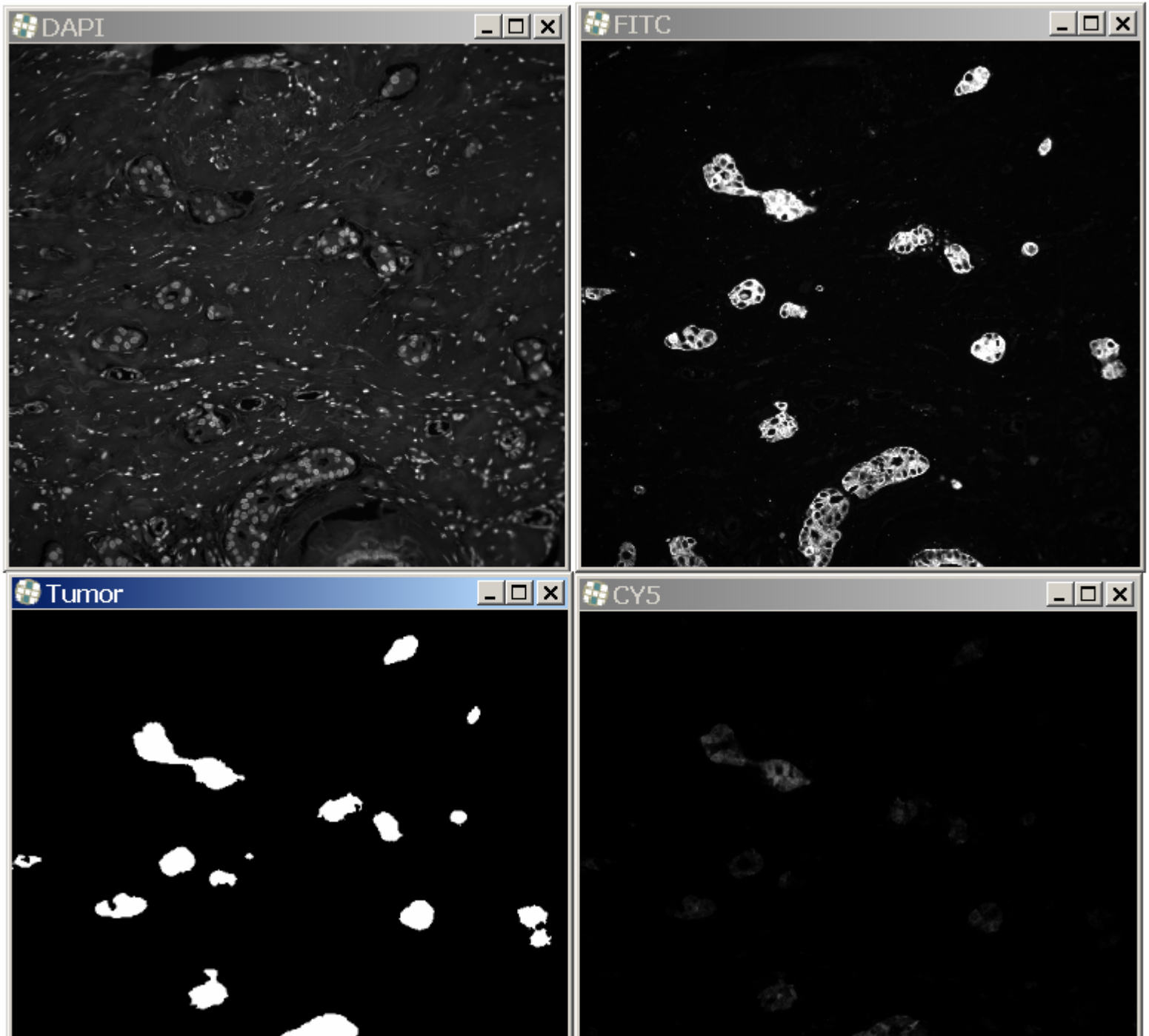



DAPI
FITC
Tumor
CY5


DAPI
FITC
Tumor
CY5


DAPI
FITC
Tumor
CY5


DAPI
FITC
Tumor
CY5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Tumor
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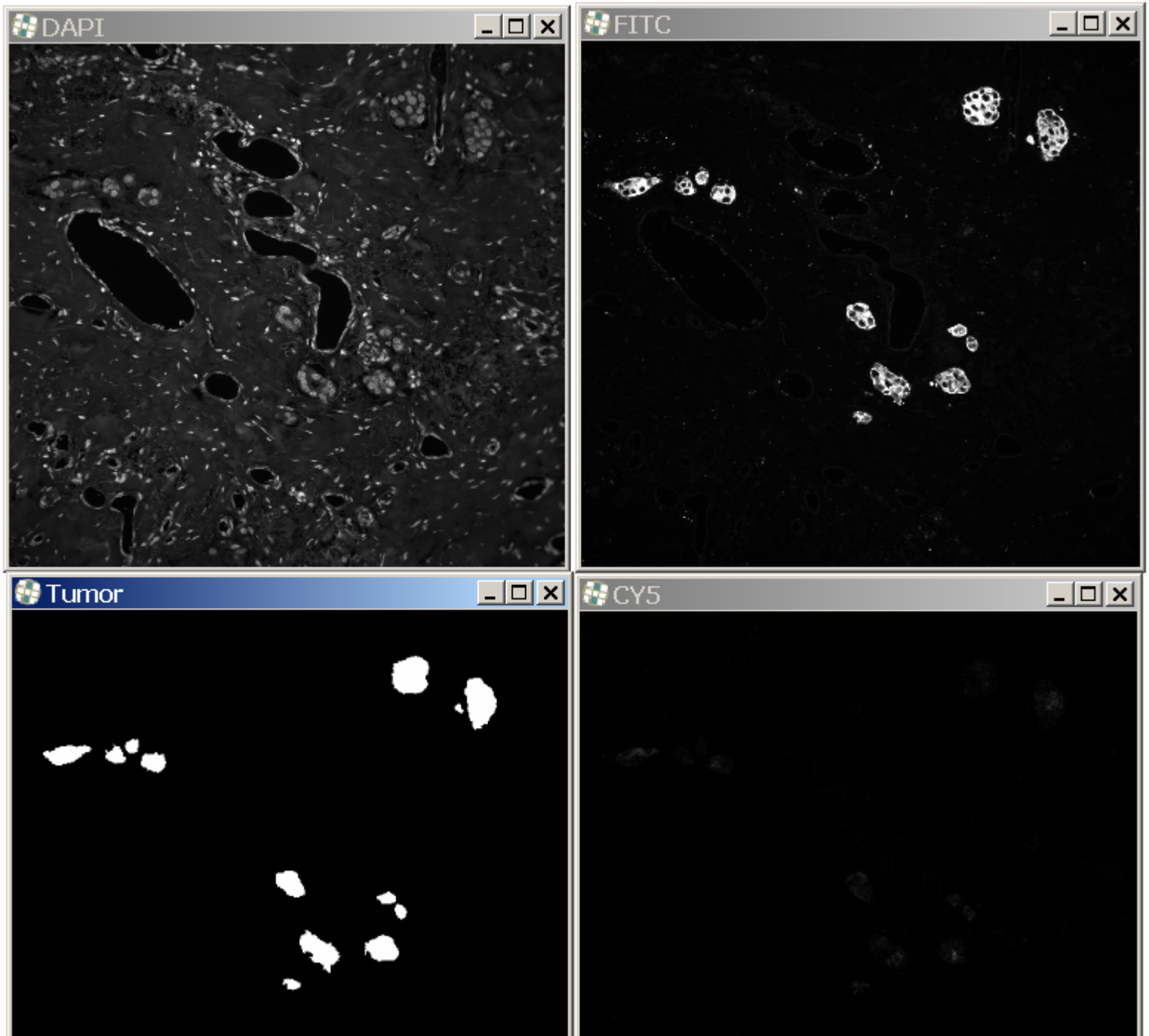
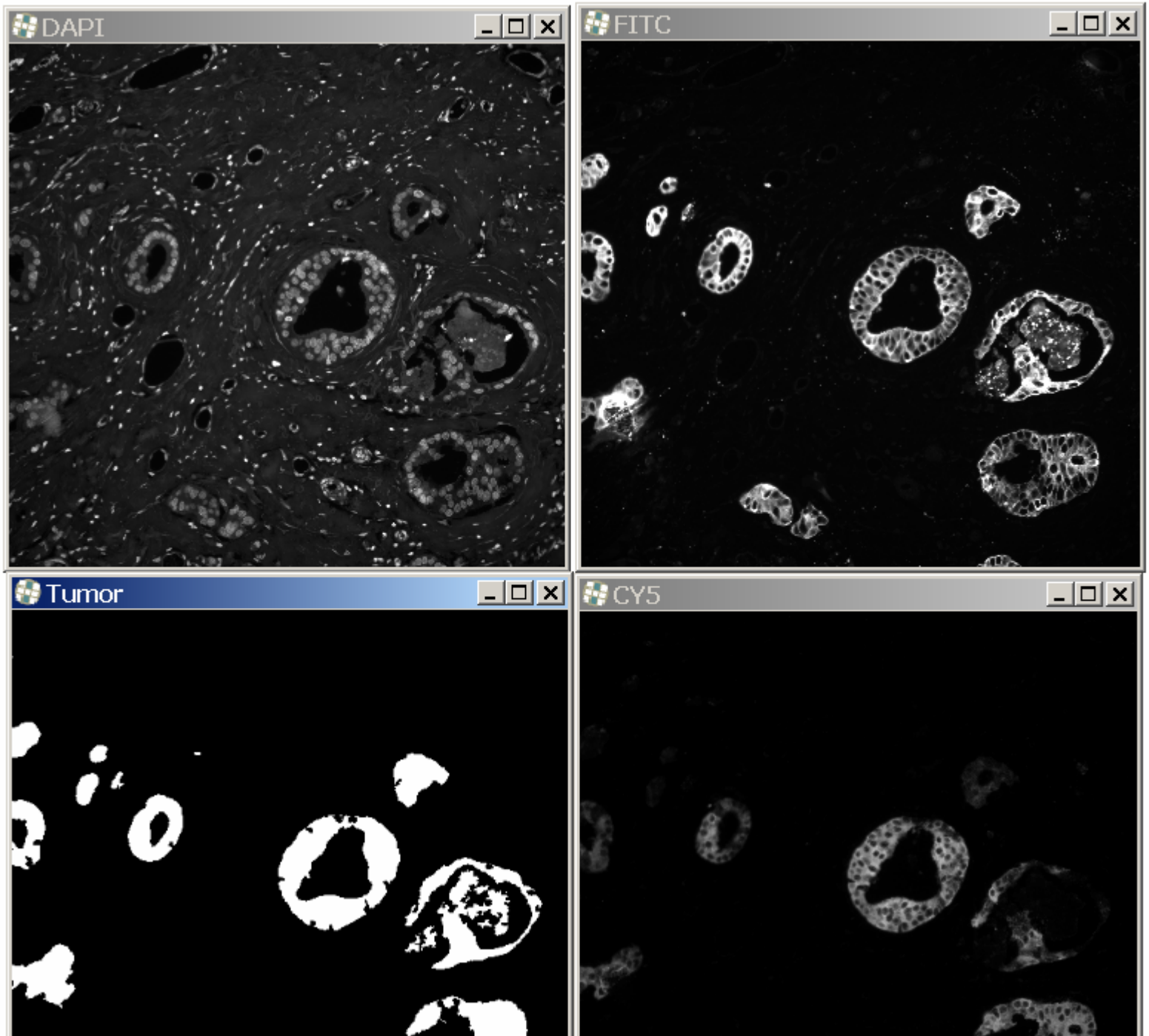

DAPI
FITC
Tumor
CY5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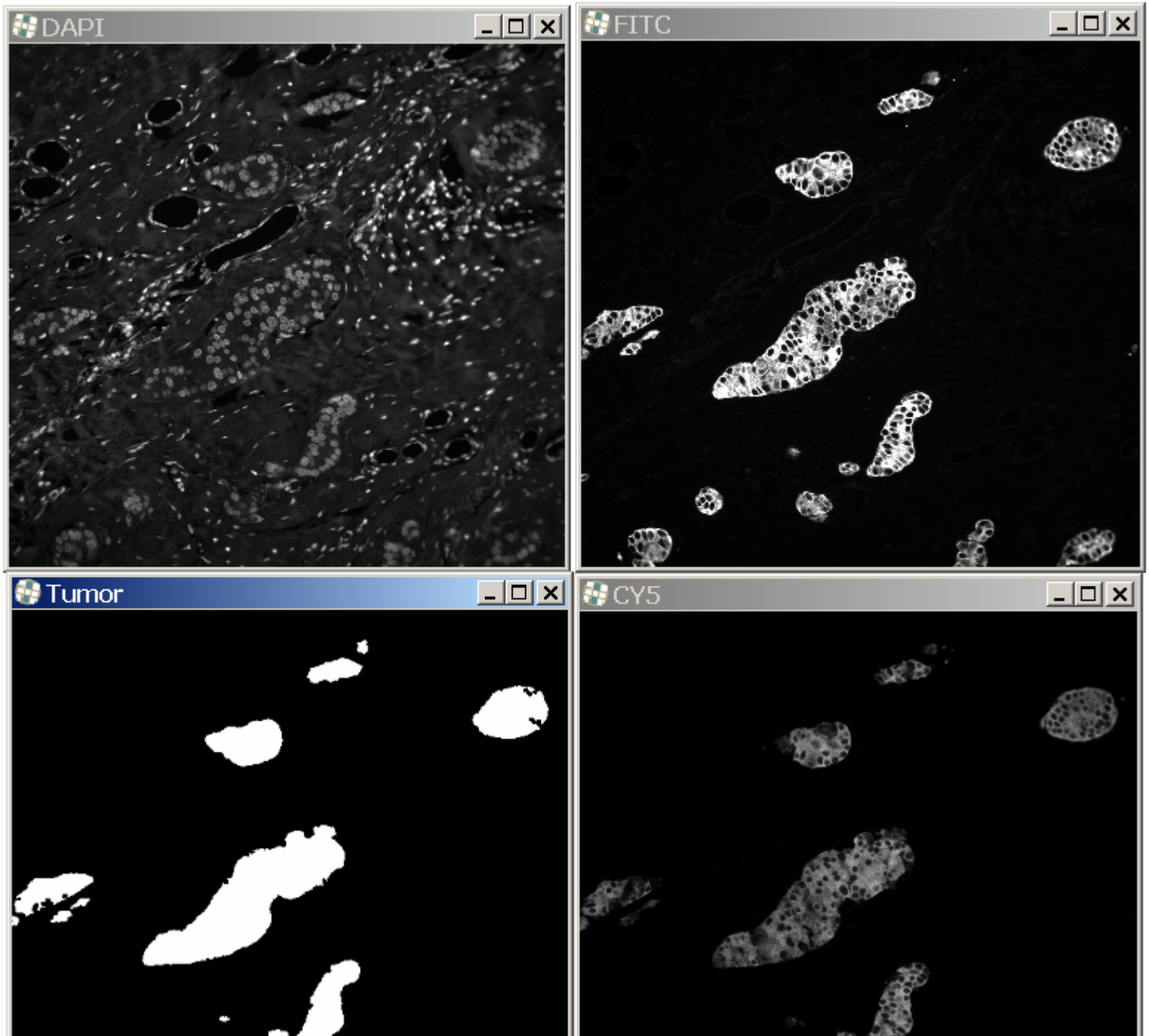
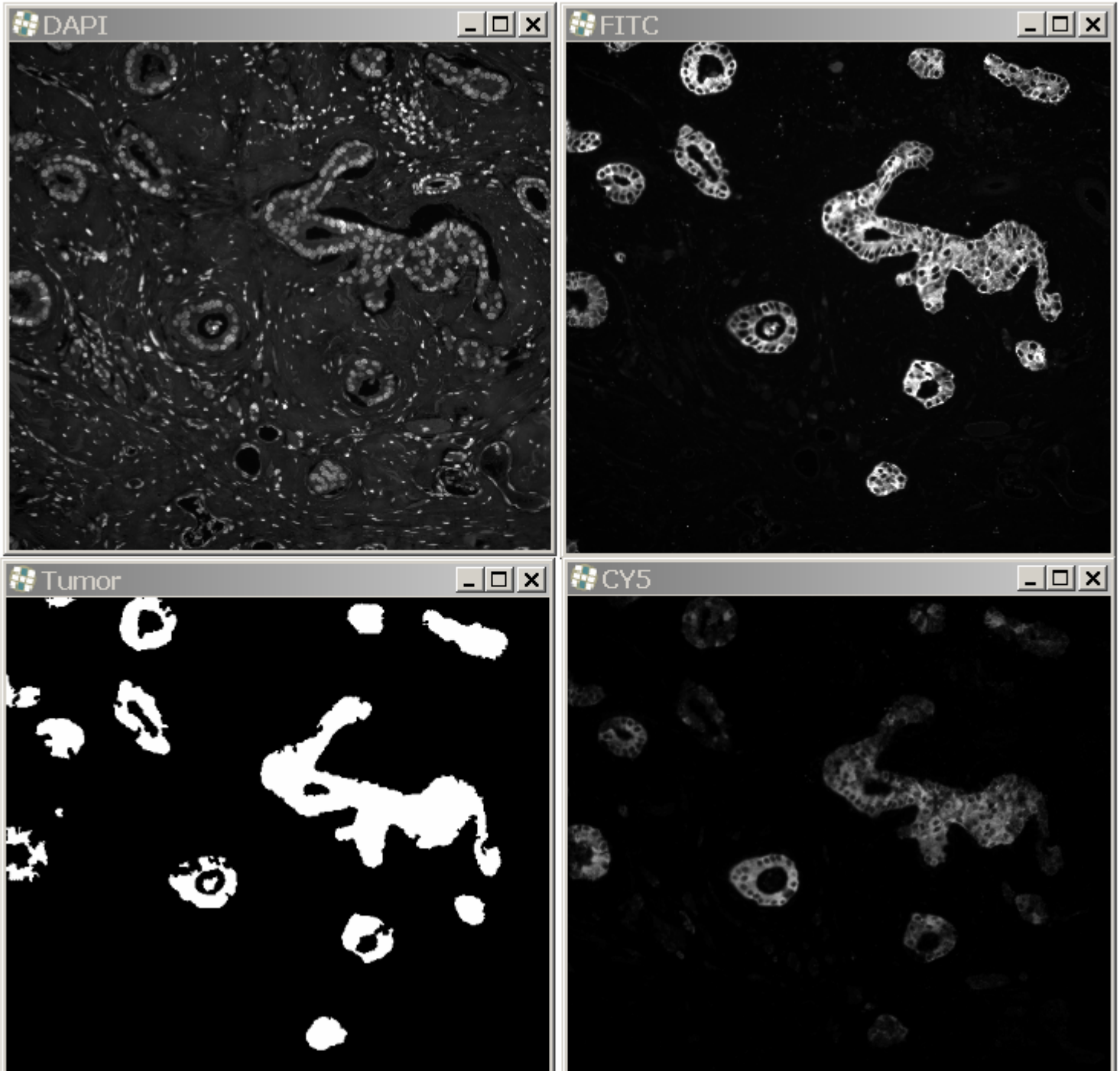
Tumor
CY5


DAPI
FITC
Tumor
CY5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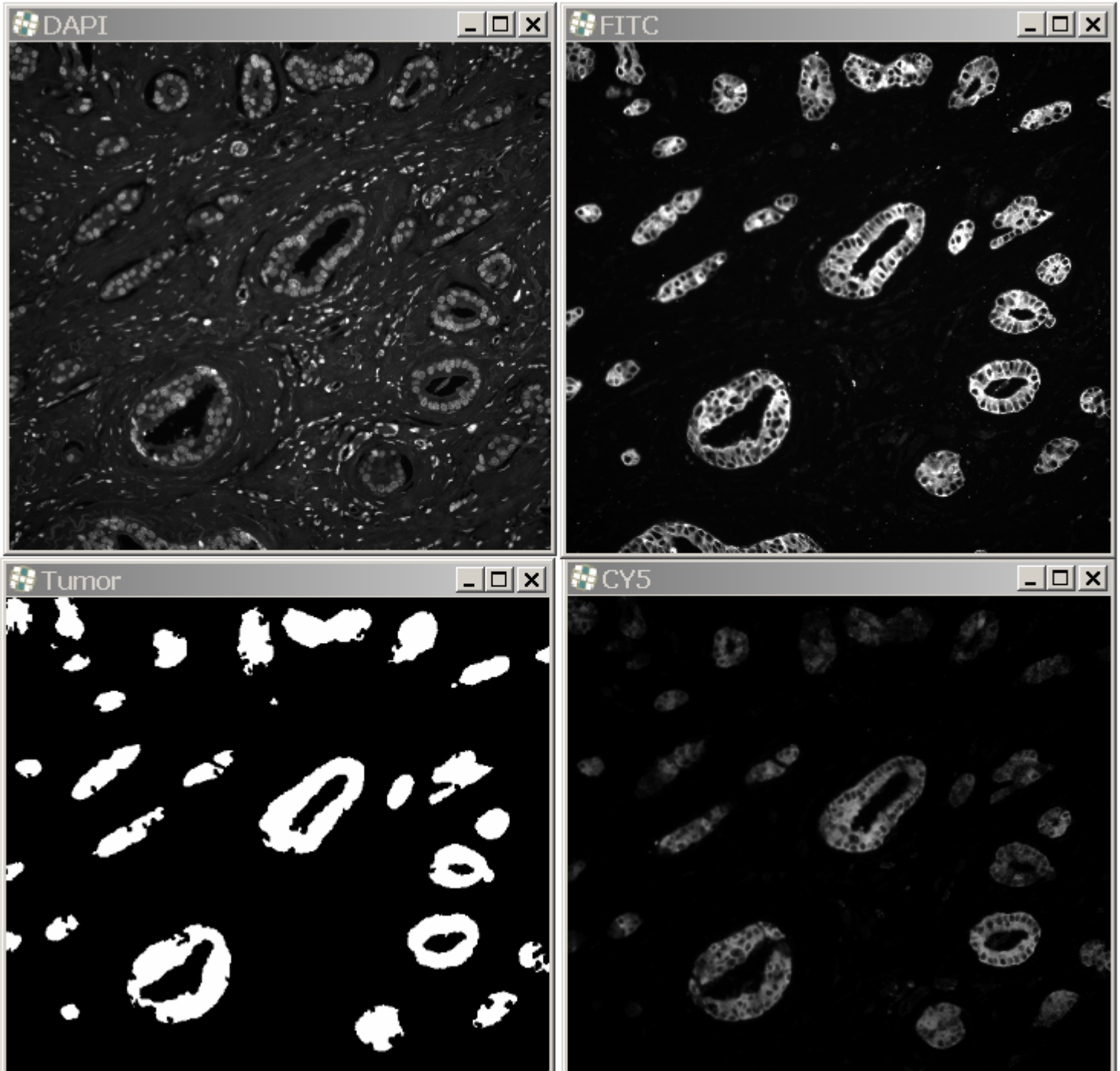
Tumor
CY5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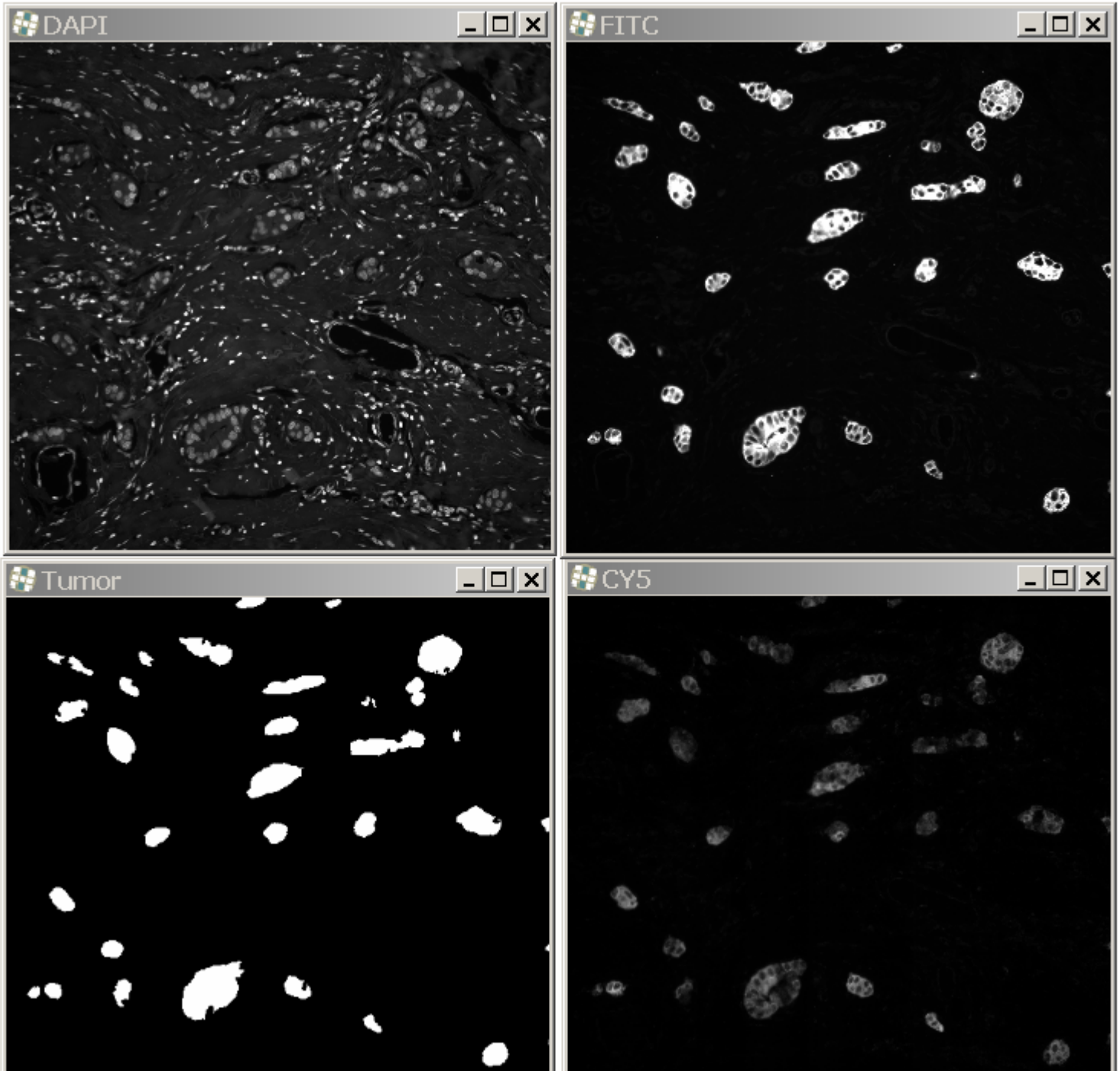
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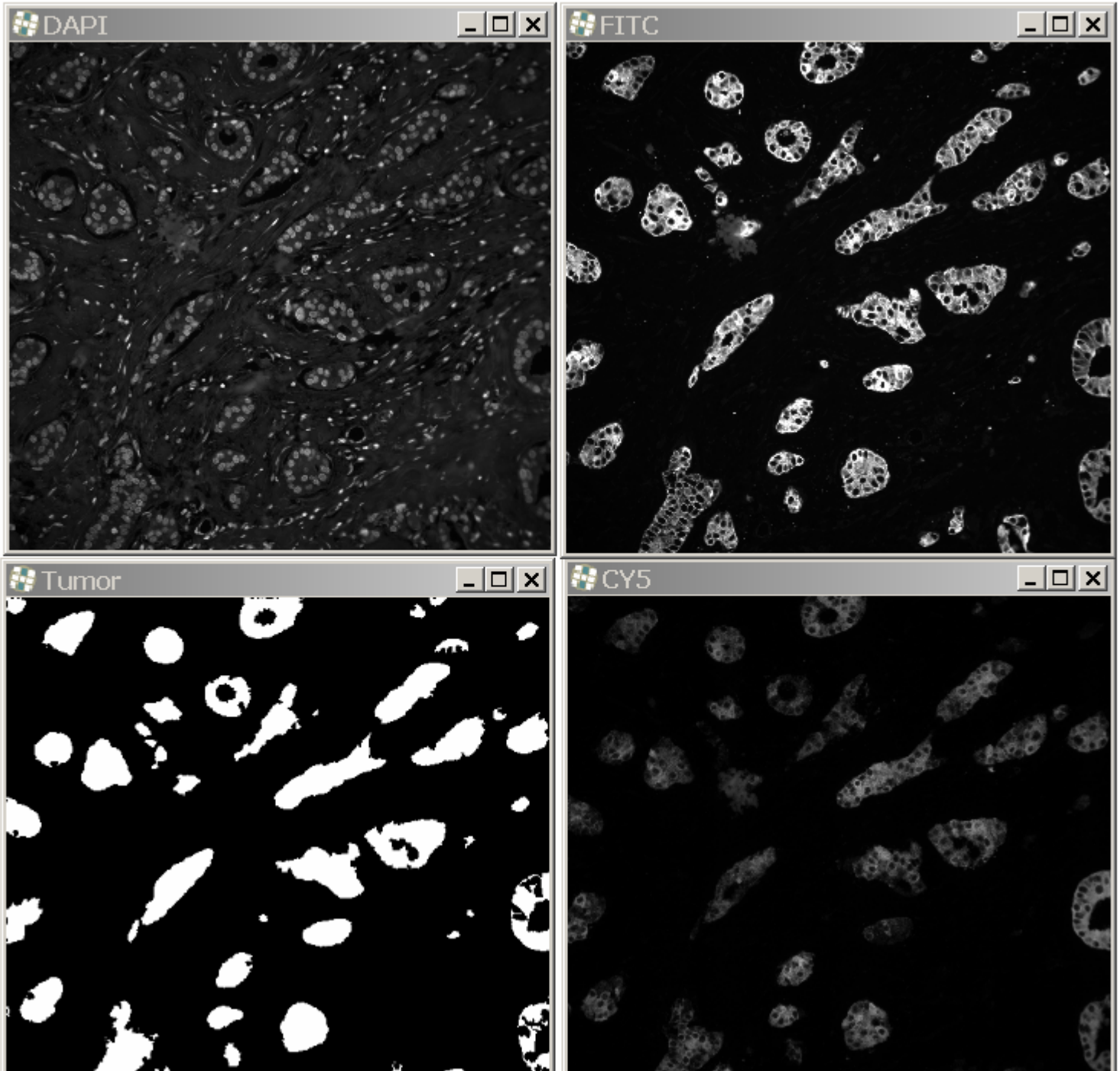
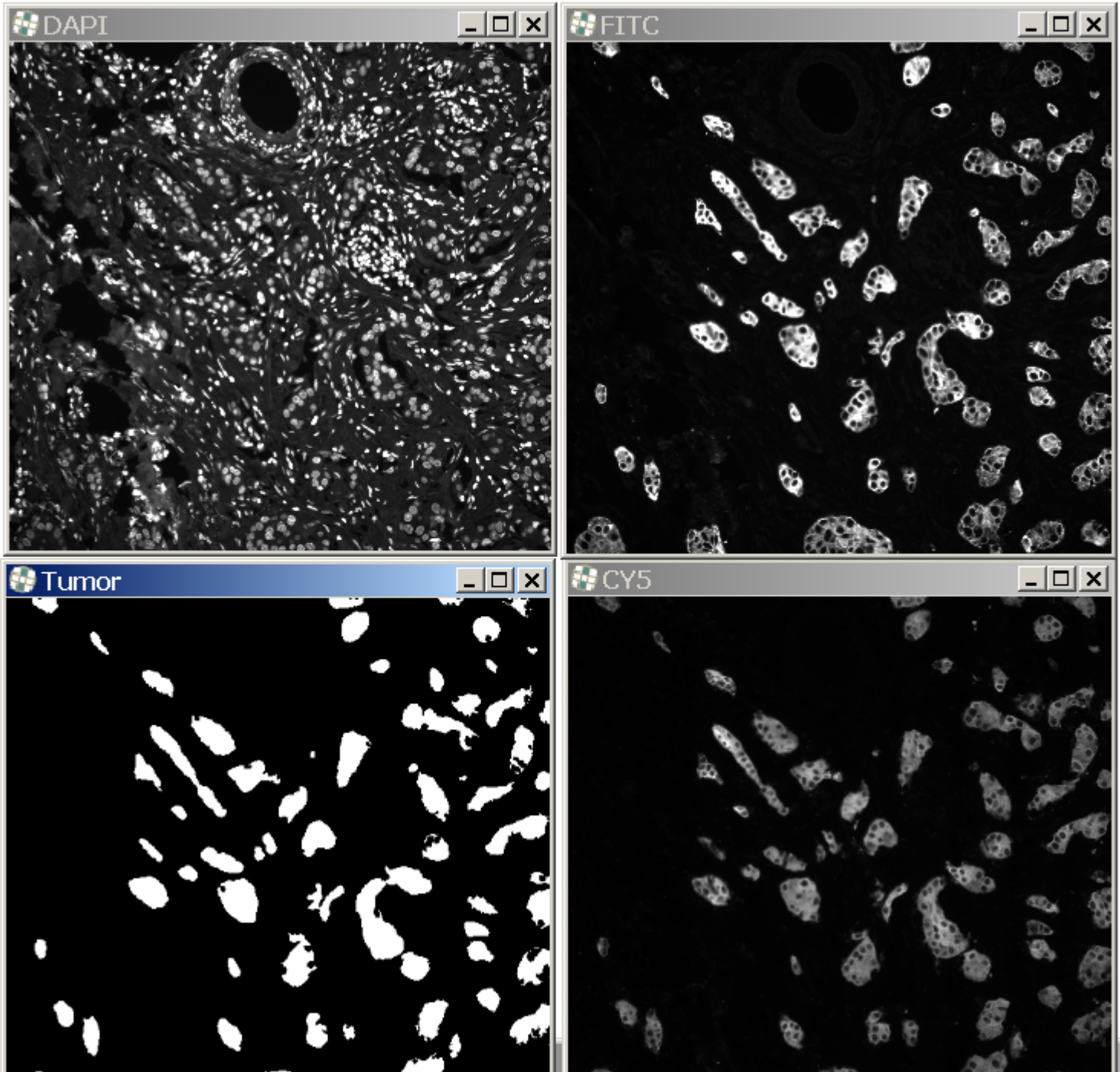
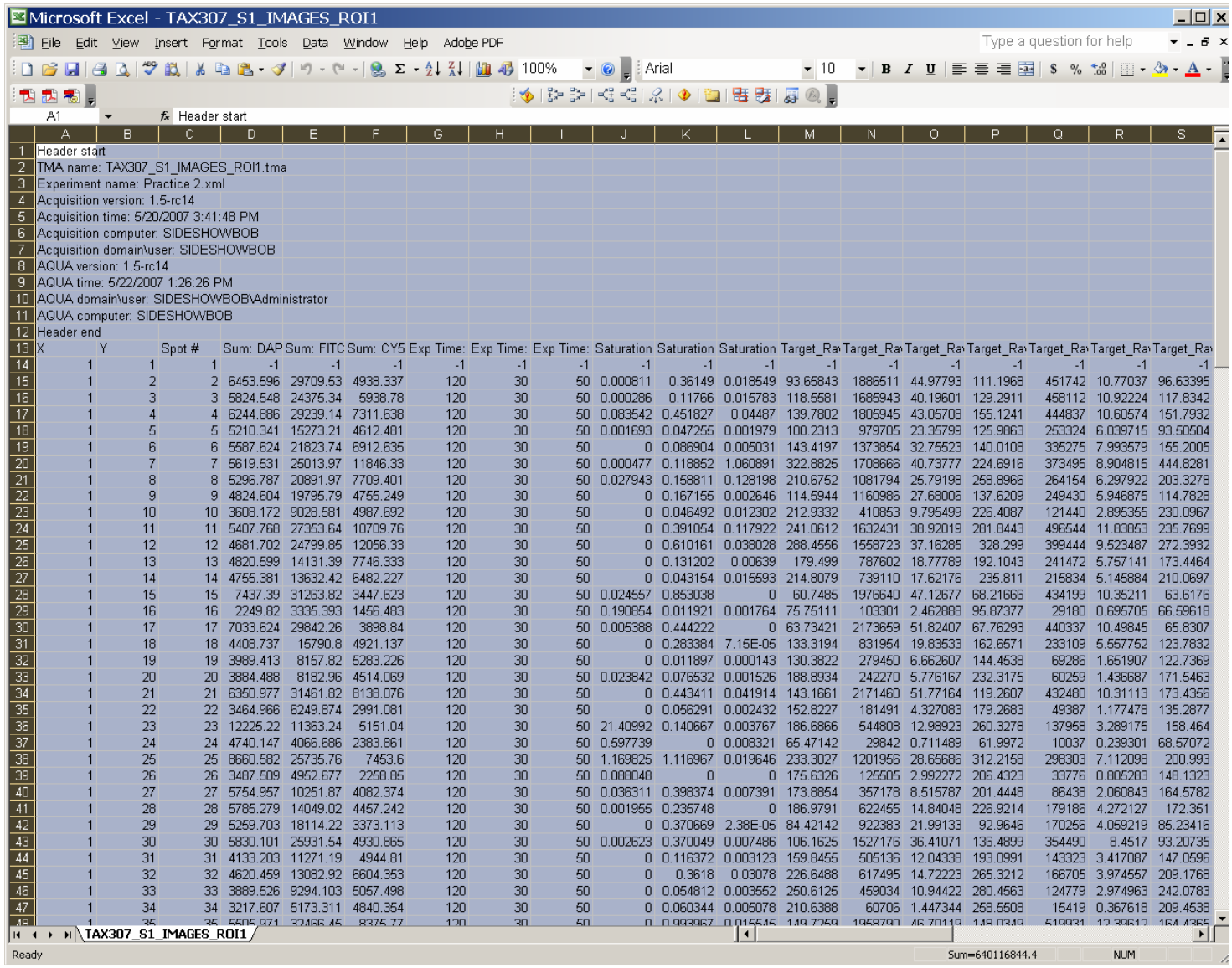
Tumor
CY5


DAPI
FITC
Tumor
CY5


DAPI
FITC
Tumor
CY5



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	Header start																		
2	TMA name: TAX307_S1_IMAGES_ROI1.tma																		
3	Experiment name: Practice 2.xml																		
4	Acquisition version: 1.5-rc14																		
5	Acquisition time: 5/20/2007 3:41:48 PM																		
6	Acquisition computer: SIDESHOWBOB																		
7	Acquisition domain\user: SIDESHOWBOB																		
8	AQUA version: 1.5-rc14																		
9	AQUA time: 5/22/2007 1:26:26 PM																		
10	AQUA domain\user: SIDESHOWBOB\Administrator																		
11	AQUA computer: SIDESHOWBOB																		
12	Header end																		
13	X	Y	Spot #	Sum: DAP	Sum: FITC	Sum: CY5	Exp Time:	Exp Time:	Exp Time:	Saturation	Saturation	Saturation	Target_Ra	Target_Ra	Target_Ra	Target_Ra	Target_Ra	Target_Ra	Target_Ra
14	1	1	1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
15	1	2	2	6453.596	29709.53	4938.337	120	30	50	0.000811	0.36149	0.018549	93.65843	1886511	44.97793	111.1968	451742	10.77037	96.63395
16	1	3	3	5824.548	24375.34	5938.78	120	30	50	0.000286	0.11766	0.015783	118.5581	1685943	40.19601	129.2911	458112	10.92224	117.8342
17	1	4	4	6244.886	29239.14	7311.638	120	30	50	0.083542	0.451827	0.04487	139.7802	1805945	43.05708	155.1241	444837	10.60574	151.7932
18	1	5	5	5210.341	15273.21	4612.481	120	30	50	0.001693	0.047255	0.001979	100.2313	979705	23.35799	125.9863	253324	6.039715	93.50504
19	1	6	6	5587.624	21823.74	6912.635	120	30	50	0	0.086904	0.005031	143.4197	1373854	32.75523	140.0108	335275	7.993579	155.2005
20	1	7	7	5619.531	25013.97	11846.33	120	30	50	0.000477	0.118852	1.060891	322.8825	1708666	40.73777	224.6916	373495	8.904815	444.8281
21	1	8	8	5296.787	20891.97	7709.401	120	30	50	0.027943	0.158811	0.128198	210.6752	1081794	25.79198	258.8966	264154	6.297922	203.3278
22	1	9	9	4824.604	19795.79	4755.249	120	30	50	0	0.167155	0.002646	114.5944	1160986	27.68006	137.6209	249430	5.946875	114.7828
23	1	10	10	3608.172	9028.581	4987.692	120	30	50	0	0.046492	0.012302	212.9332	410853	9.795499	226.4087	121440	2.895355	230.0967
24	1	11	11	5407.768	27353.64	10709.76	120	30	50	0	0.391054	0.117922	241.0612	1632431	38.92019	281.8443	496544	11.83853	235.7699
25	1	12	12	4681.702	24799.85	12056.33	120	30	50	0	0.610161	0.038028	288.4556	1558723	37.16285	328.299	399444	9.523487	272.3932
26	1	13	13	4820.599	14131.39	7746.333	120	30	50	0	0.131202	0.00639	179.499	787602	18.77789	192.1043	241472	5.757141	173.4464
27	1	14	14	4755.381	13632.42	6482.227	120	30	50	0	0.043154	0.015593	214.8079	739110	17.62176	235.811	215834	5.145884	210.0697
28	1	15	15	7437.39	31263.82	3447.623	120	30	50	0.024557	0.853038	0	60.7485	1976640	47.12677	68.21666	434199	10.35211	63.6176
29	1	16	16	2249.82	3335.393	1456.483	120	30	50	0.190854	0.011921	0.001764	75.75111	103301	2.462888	95.87377	29180	0.695705	66.59618
30	1	17	17	7033.624	29842.26	3898.84	120	30	50	0.005388	0.444222	0	63.73421	2173659	51.82407	67.76293	440337	10.49845	65.8307
31	1	18	18	4408.737	15790.8	4921.137	120	30	50	0	0.283384	7.15E-05	133.3194	831954	19.83533	162.6571	233109	5.557752	123.7832
32	1	19	19	3989.413	8157.82	5283.226	120	30	50	0	0.011897	0.000143	130.3822	279450	6.662607	144.4538	69286	1.651907	122.7369
33	1	20	20	3884.488	8182.96	4514.069	120	30	50	0.023842	0.076532	0.001526	188.8934	242270	5.776167	232.3175	60259	1.436687	171.5463
34	1	21	21	6350.977	31461.82	8138.076	120	30	50	0	0.443411	0.041914	143.1661	2171460	51.77164	119.2607	432480	10.31113	173.4356
35	1	22	22	3464.966	6249.874	2991.081	120	30	50	0	0.056291	0.002432	152.8227	181491	4.327083	179.2683	49387	1.177478	135.2877
36	1	23	23	12225.22	11363.24	5151.04	120	30	50	21.40992	0.140667	0.003767	186.6866	544808	12.98923	260.3278	137958	3.289175	158.464
37	1	24	24	4740.147	4066.686	2383.861	120	30	50	0.597739	0	0.008321	65.47142	29842	0.711489	61.9972	10037	0.239301	68.57072
38	1	25	25	8660.582	25735.76	7453.6	120	30	50	1.169825	1.116967	0.019646	233.3027	1201956	28.65686	312.2158	298303	7.112098	200.993
39	1	26	26	3487.509	4952.677	2258.85	120	30	50	0.088048	0	0	175.6326	125505	2.992272	206.4323	33776	0.805283	148.1323
40	1	27	27	5754.957	10251.87	4082.374	120	30	50	0.036311	0.398374	0.007391	173.8854	357178	8.515787	201.4448	86438	2.060843	164.5782
41	1	28	28	5785.279	14049.02	4457.242	120	30	50	0.001955	0.235748	0	186.9791	622455	14.84048	226.9214	179186	4.272127	172.351
42	1	29	29	5259.703	18114.22	3373.113	120	30	50	0	0.370669	2.38E-05	84.42142	922383	21.99133	92.9646	170256	4.059219	85.23416
43	1	30	30	5830.101	25931.54	4930.865	120	30	50	0.002623	0.370049	0.007486	106.1625	1527176	36.41071	136.4899	354490	8.4517	93.20735
44	1	31	31	4133.203	11271.19	4944.81	120	30	50	0	0.116372	0.003123	159.8455	505136	12.04338	193.0991	143323	3.417087	147.0596
45	1	32	32	4620.459	13082.92	6604.353	120	30	50	0	0.3618	0.03078	226.6488	617495	14.72223	265.3212	166705	3.974557	209.1768
46	1	33	33	3889.526	9294.103	5057.498	120	30	50	0	0.054812	0.003552	250.6125	459034	10.94422	280.4563	124779	2.974963	242.0783
47	1	34	34	3217.607	5173.311	4840.354	120	30	50	0	0.060344	0.005078	210.6388	60706	1.447344	258.5508	15419	0.367618	209.4538
48	1	35	35	5505.971	32466.45	8375.77	120	30	50	0	0.993967	0.015546	149.7259	1958790	46.70119	148.0349	519931	12.39612	164.4365

Analysis Data Spreadsheet

Saturation %: DAPI	Saturation %: FITC	Saturation %: CY5	Target_Raw in Tumor AQI	Target_Raw in Tumor (	Target_Raw in Tumor (	Target_Raw in Nucleus	Target_Raw in Nucleus	Target_Raw in Nucleus
-1	-1	-1	-1	-1	-1	-1	-1	-1
0.00081062	0.36149025	0.01854897	93.65843201	1886511	44.97792816	111.1968079	451742	10.77036858
0.0002861	0.11765957	0.01578331	118.5581207	1685943	40.1960144	129.2911072	458112	10.92224121
0.08354187	0.45182705	0.04487038	139.7801514	1805945	43.05708313	155.1240692	444837	10.60574055
0.00169277	0.04725456	0.00197887	100.231308	979705	23.35798645	125.9863052	253324	6.03971481
0	0.08690357	0.00503063	143.419693	1373854	32.75523376	140.0108032	335275	7.99357891
0.00047684	0.11885166	1.06089115	322.8825378	1708666	40.73777008	224.6916351	373495	8.90481472
0.02794266	0.15881062	0.12819767	210.6752014	1081794	25.79197884	258.896637	264154	6.29792213
0	0.16715527	0.00264645	114.5944443	1160986	27.68006325	137.6209412	249430	5.94687462
0	0.04649162	0.0123024	212.933197	410853	9.79549885	226.4087372	121440	2.89535522
0	0.39105415	0.11792183	241.0612488	1632431	38.9201889	281.8442688	496544	11.83853149
0	0.61016083	0.03802776	288.4555969	1558723	37.16285324	328.2989502	399444	9.52348709
0	0.13120174	0.00638962	179.4990387	787602	18.77789497	192.1043091	241472	5.75714111
0	0.04315376	0.01559258	214.8079224	739110	17.6217556	235.8109894	215834	5.14588356
0.02455711	0.85303783	0	60.74849701	1976640	47.12677002	68.21665955	434199	10.35211086
0.19085407	0.01192093	0.0017643	75.75110626	103301	2.46288776	95.87377167	29180	0.69570541
0.00538826	0.4442215	0	63.73421478	2173659	51.82406998	67.76293182	440337	10.49845219
0	0.28338432	0.00007153	133.3193665	831954	19.83532906	162.6570892	233109	5.55775166
0	0.01189709	0.00014305	130.3822479	279450	6.66260719	144.4538117	69286	1.65190697
0.02384186	0.07653236	0.00152588	188.8933563	242270	5.77616692	232.3175049	60259	1.43668652
0	0.44341087	0.04191399	143.1661224	2171460	51.77164078	119.2606659	432480	10.31112671
0	0.05629063	0.00243187	152.8227234	181491	4.32708263	179.2683258	49387	1.17747784
21.40991592	0.14066696	0.00376701	186.6865692	544808	12.98923492	260.3277893	137958	3.28917503
0.59773922	0	0.00832081	65.47142029	29842	0.71148872	61.9971962	10037	0.23930073
1.1698246	1.1169672	0.01964569	233.3026581	1201956	28.65686417	312.2158203	298303	7.11209774
0.08804798	0	0	175.6326294	125505	2.99227238	206.4323273	33776	0.80528259
0.03631115	0.3983736	0.00739098	173.8853607	357178	8.51578712	201.4448242	86438	2.06084251
0.00195503	0.23574829	0	186.9790649	622455	14.84048367	226.9214325	179186	4.27212715
0	0.37066936	0.00002384	84.42141724	922383	21.99132538	92.96459961	170256	4.05921936
0.0026226	0.37004948	0.00748634	106.1624832	1527176	36.4107132	136.4899445	354490	8.45170021
0	0.11637211	0.00312328	159.845459	505136	12.04338074	193.0991364	143323	3.4170866
0	0.36180019	0.03077984	226.6488037	617495	14.72222805	265.321228	166705	3.97455692
0	0.05481243	0.00355244	250.6124573	459034	10.9442234	280.4563294	124779	2.97496319
0	0.06034374	0.00507832	210.6387634	60706	1.44734383	258.5507813	15419	0.36761761
0	0.99396706	0.01554489	149.7258606	1958790	46.70119476	148.0349274	519931	12.39612103

TAX307_S1_IMAGES_ROI1

eady

NUM

Data Analysis

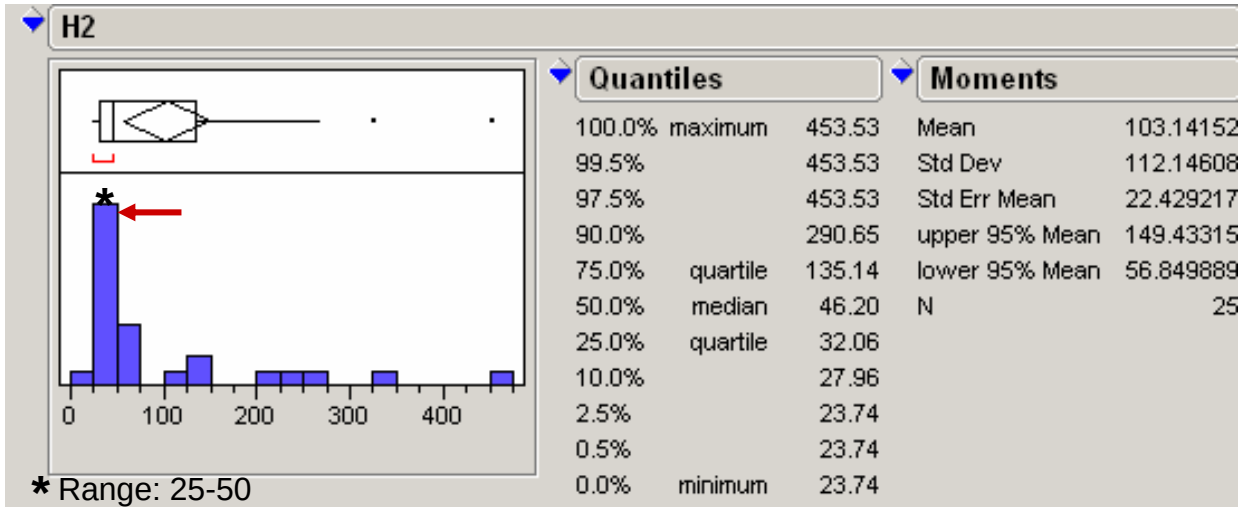
- 15 histograms from beginning, middle, end of cohort for AQUA score distribution

- Coding of all cases:
- Invasive
- Mixed DCIS + Invasive
- No tumor, normal ducts present
- Stroma, no ducts, no tumor
- Blank, no tissue
- Technical artifact (blurry, lint, etc)
- Other
- Show Pathologist

Appendix F

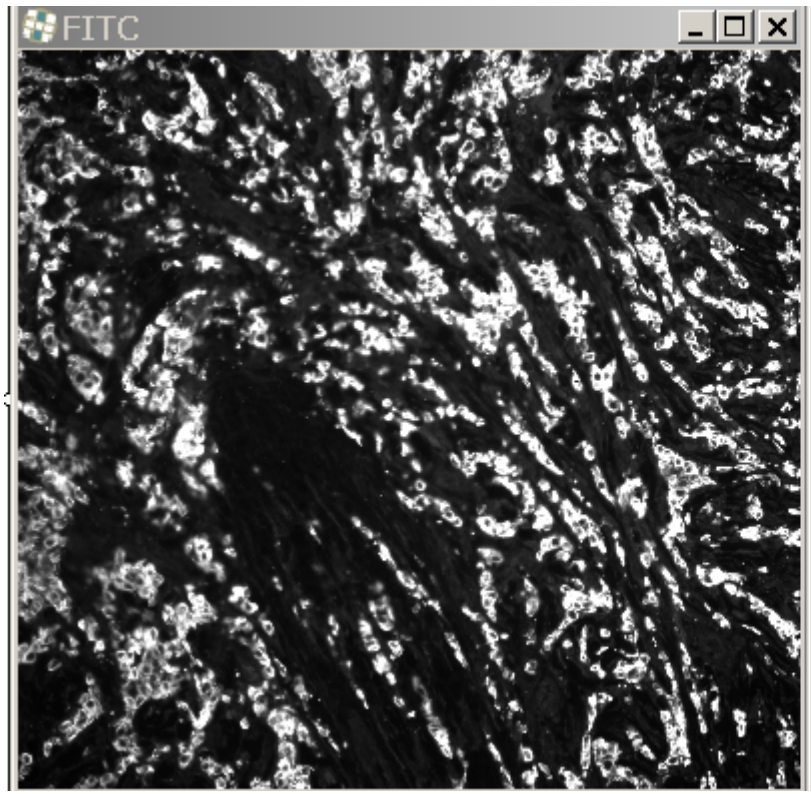
Case: H2.1

AQUA
Score: 40.68

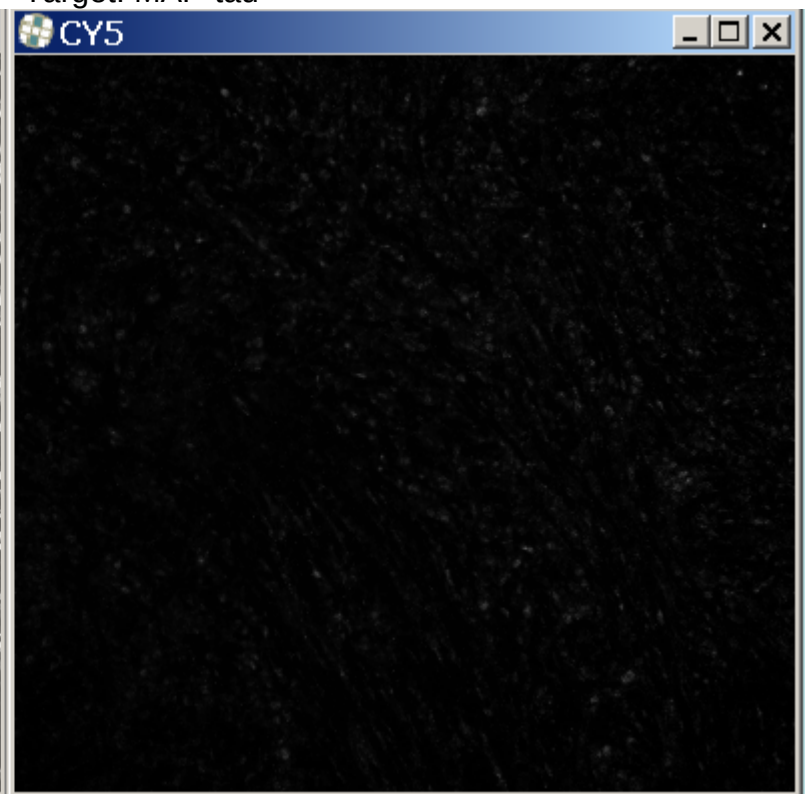


* Range: 25-50

Tumor Mask

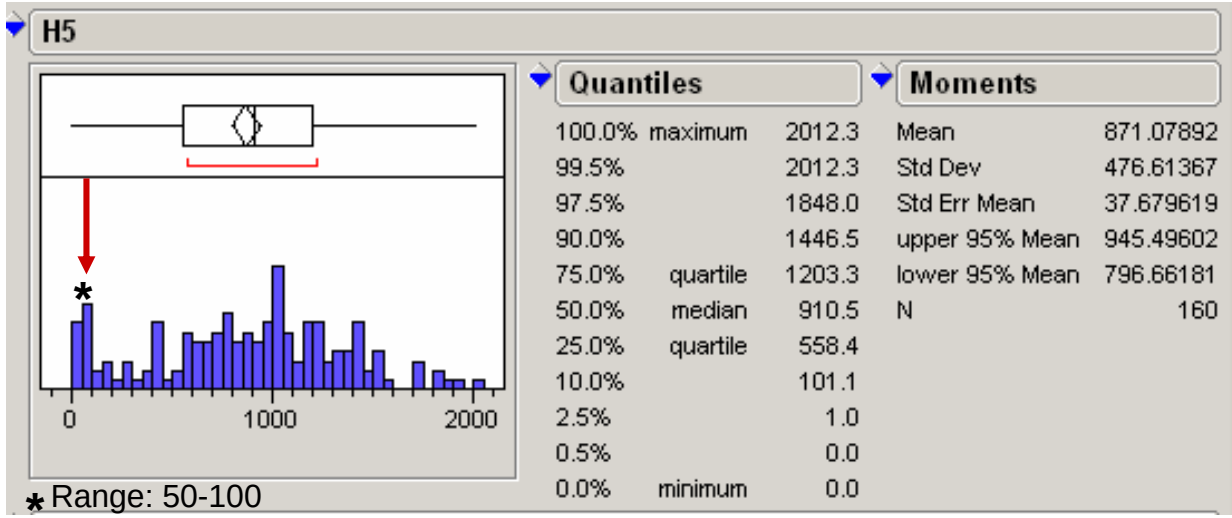


Target: MAP-tau

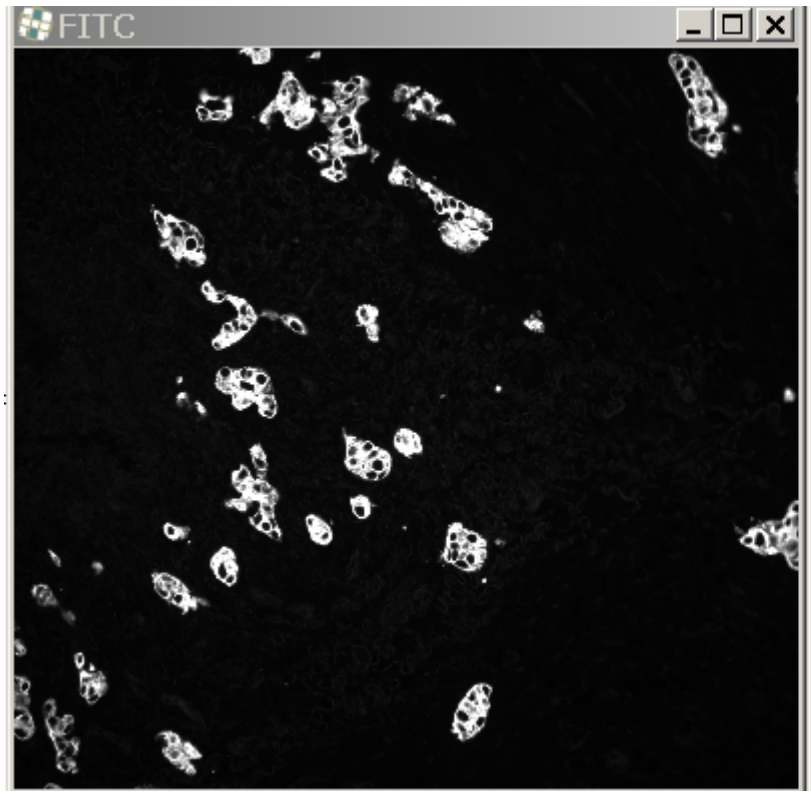


Case: H5.2

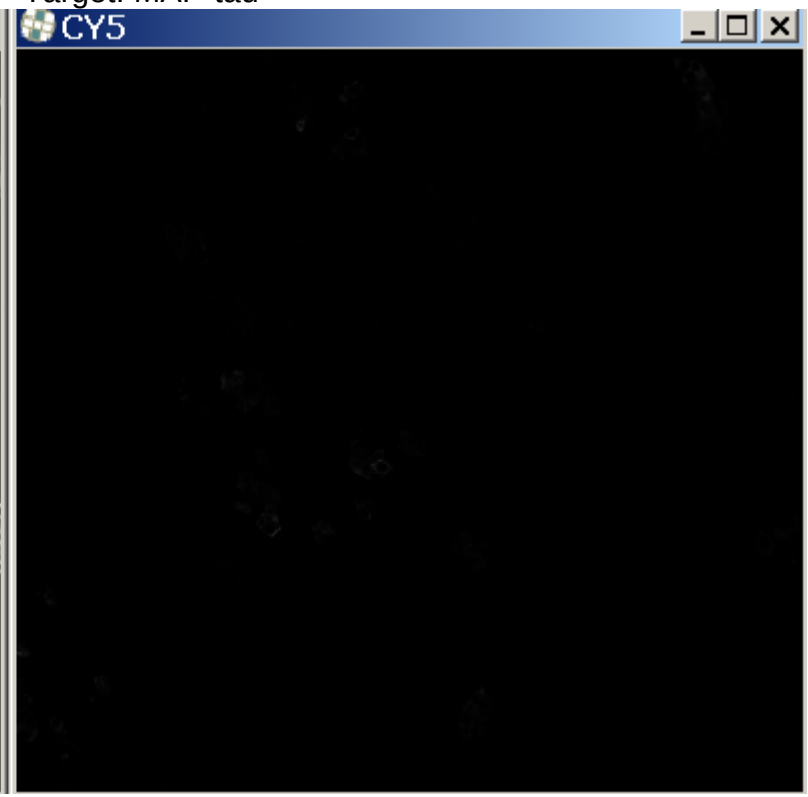
AQUA
Score: 63.77



Tumor Mask

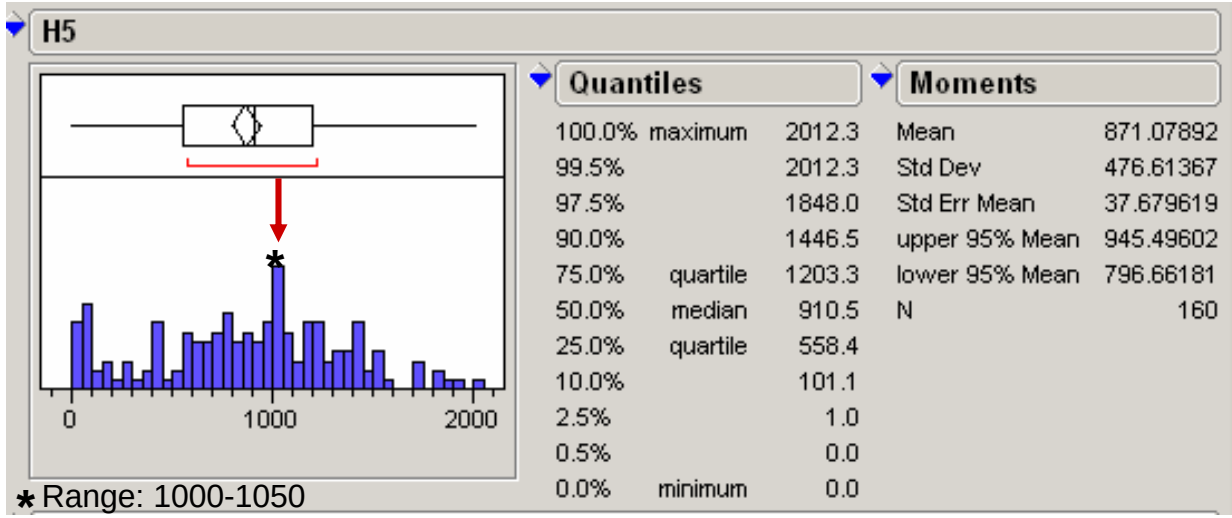


Target: MAP-tau

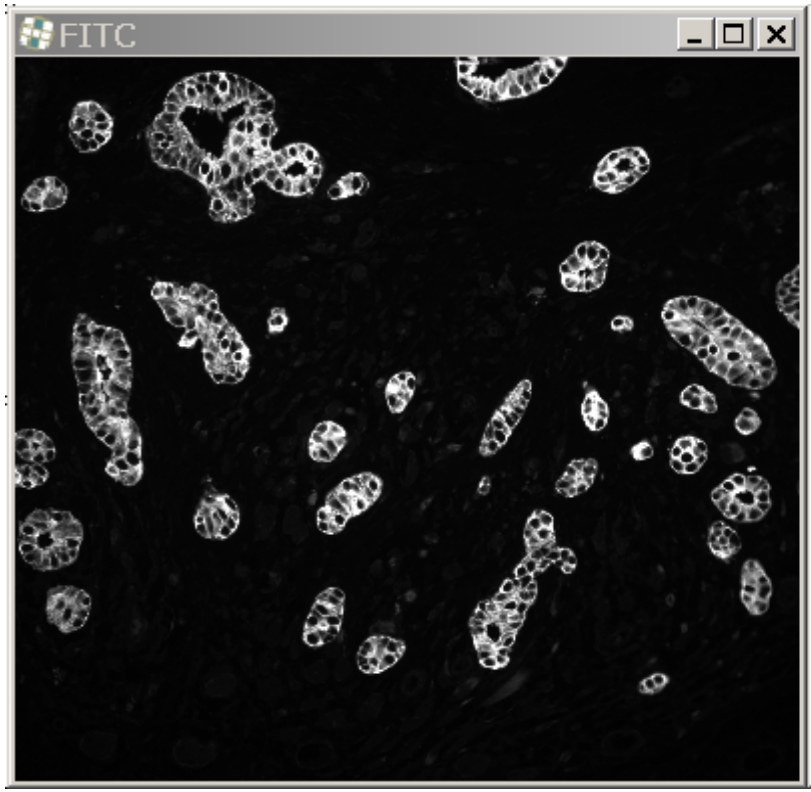


Case: H5.3

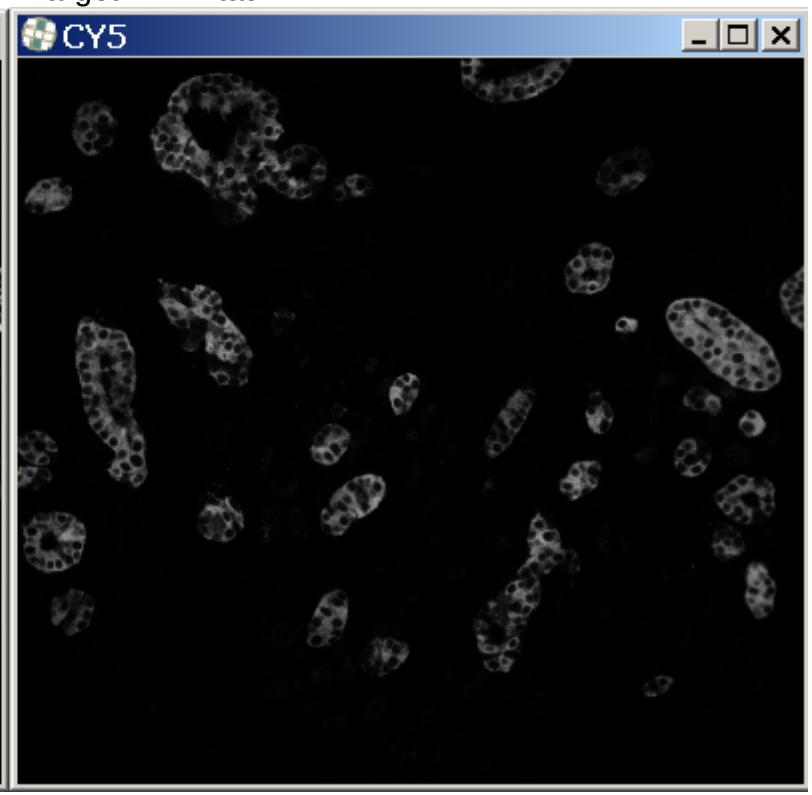
AQUA
Score: 1020.77



Tumor Mask

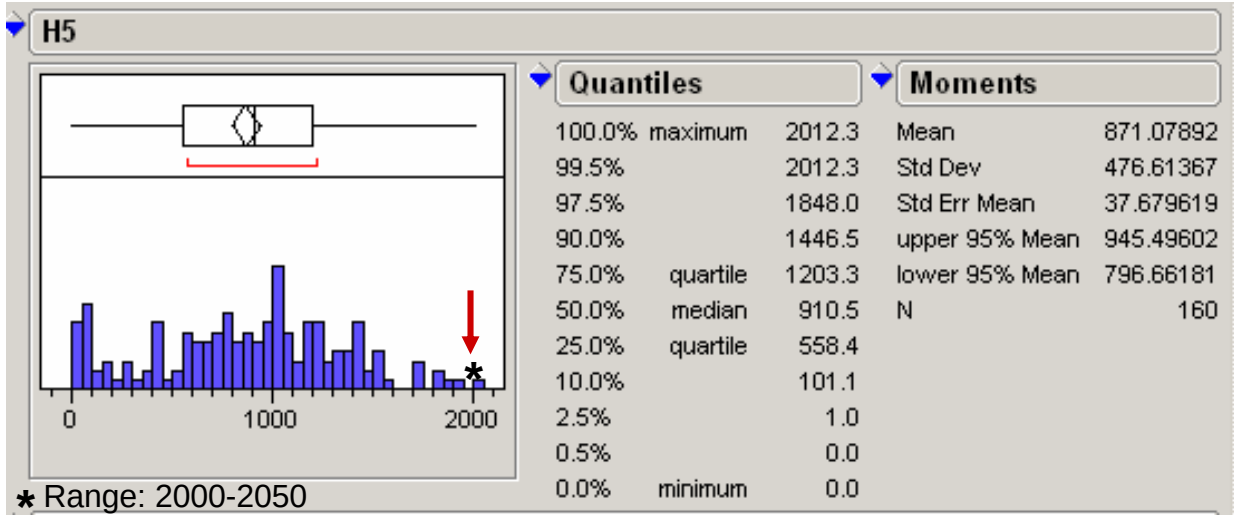


Target: MAP-tau

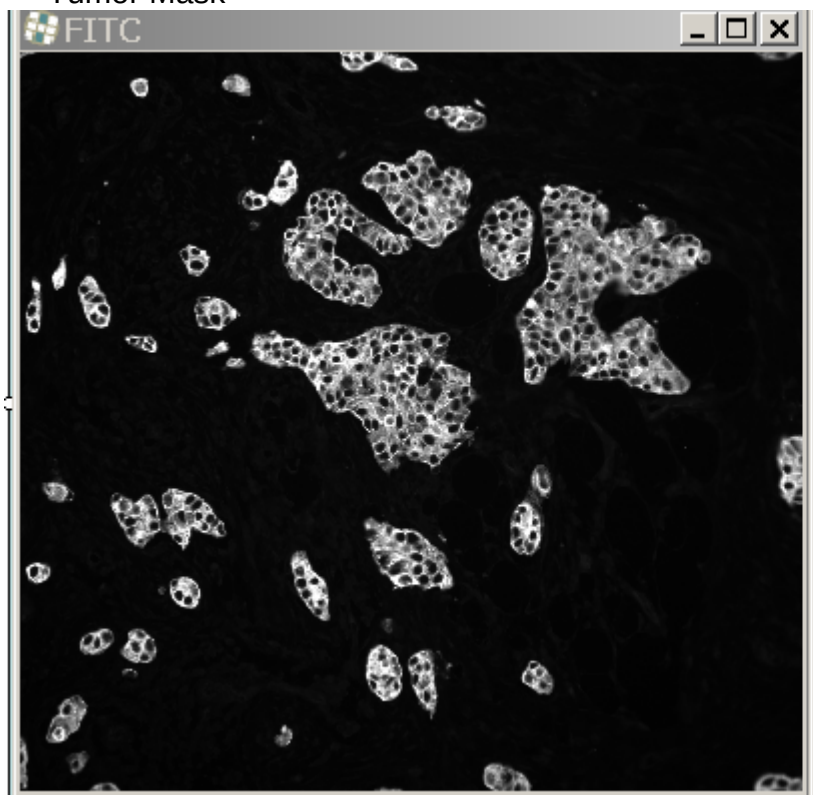


Case: H5.4

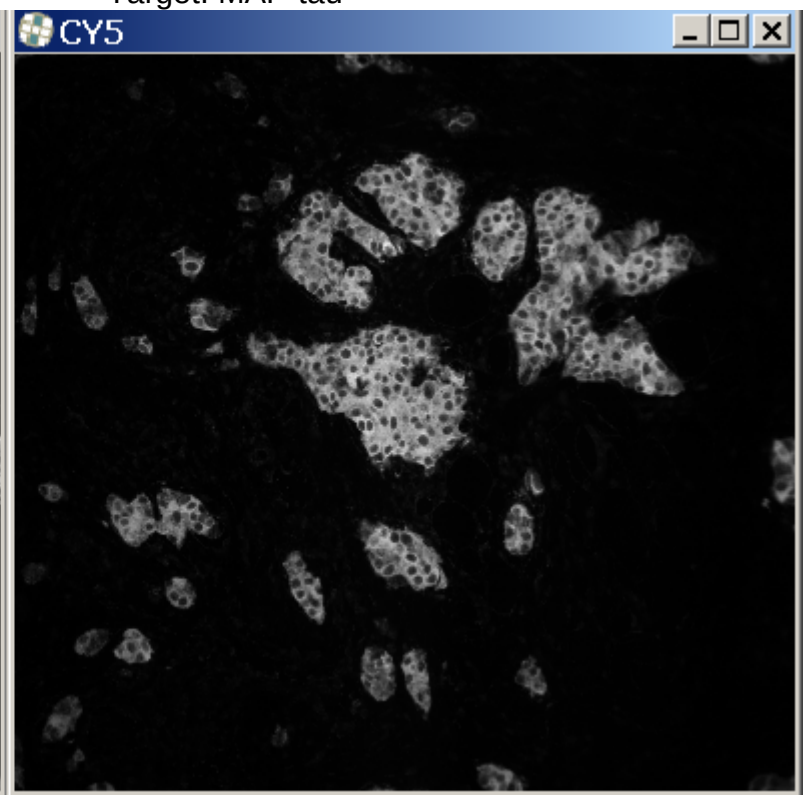
AQUA
Score: 2012.27



Tumor Mask

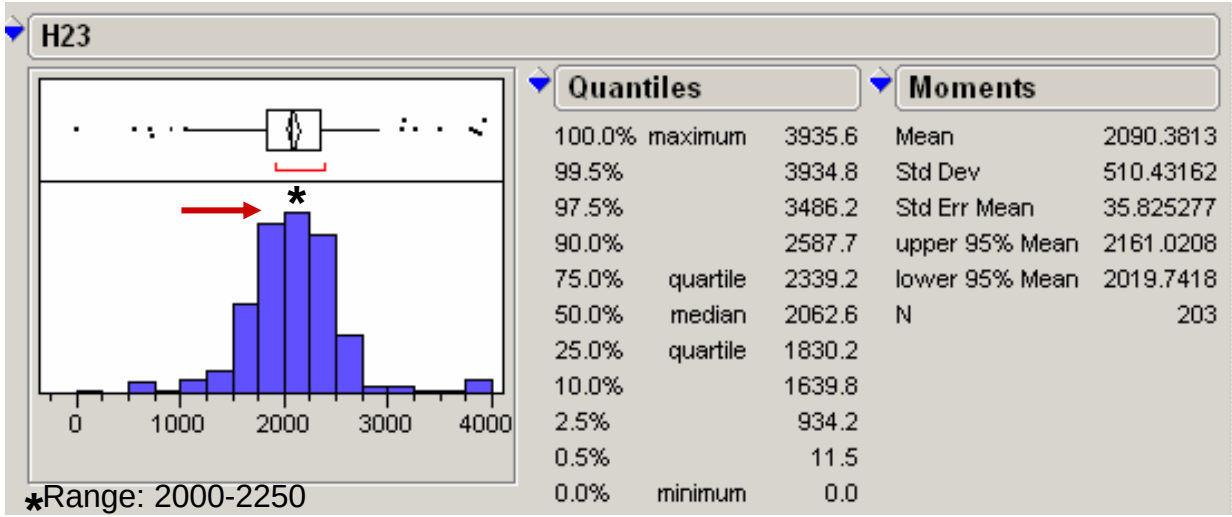


Target: MAP-tau



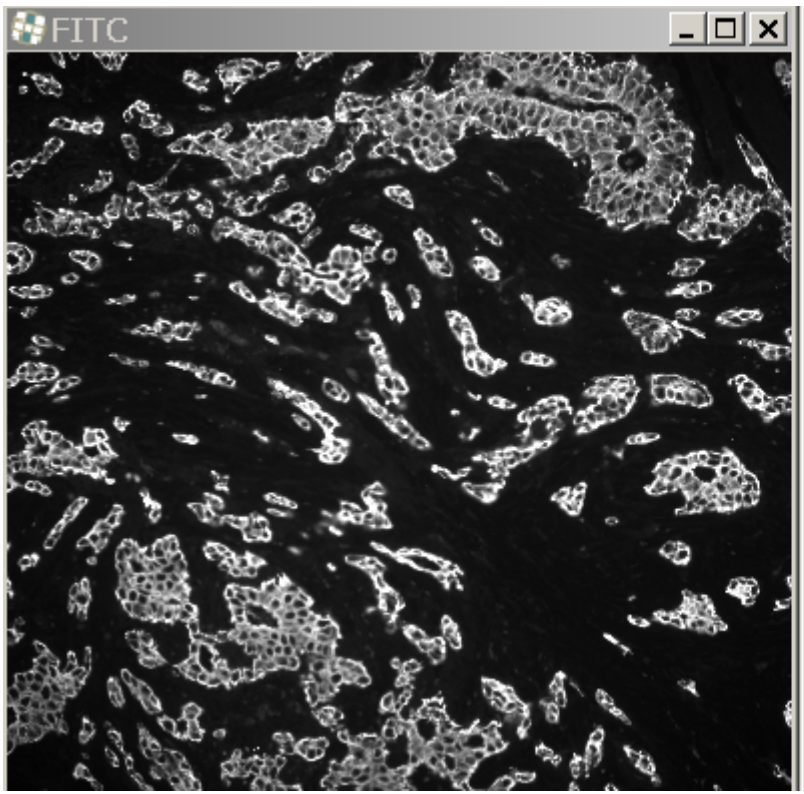
Case: H23.5

AQUA
Score: 2239.94

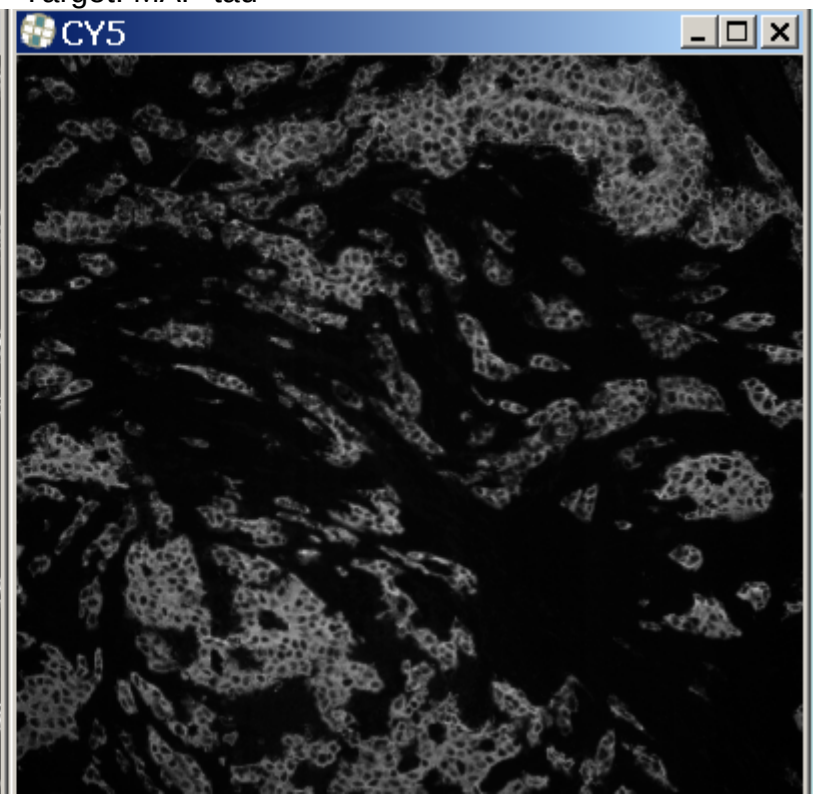


*Range: 2000-2250

Tumor Mask

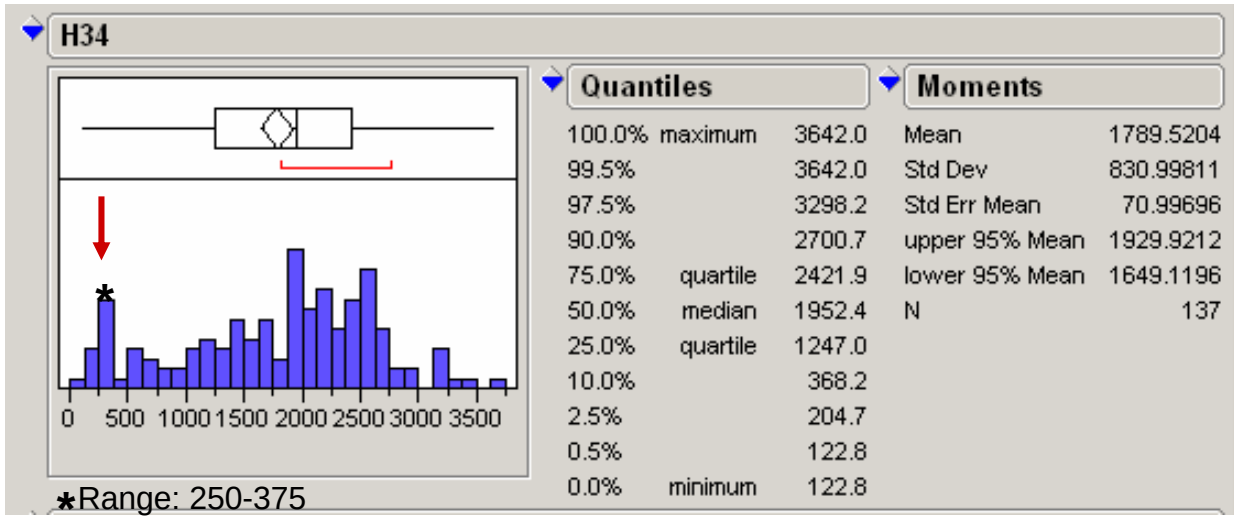


Target: MAP-tau

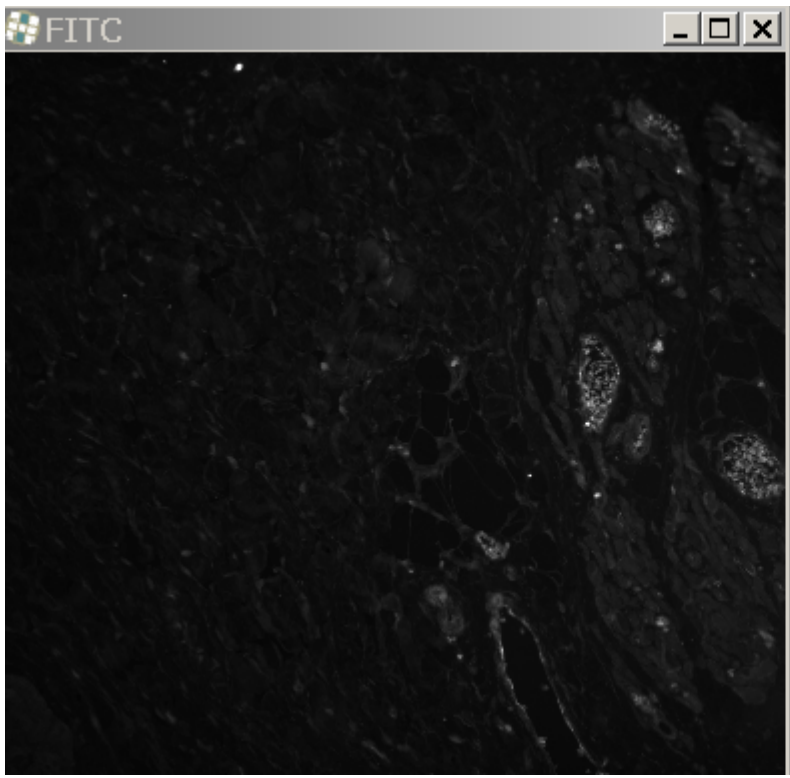


Case: H34.6

AQUA
Score: 277.46



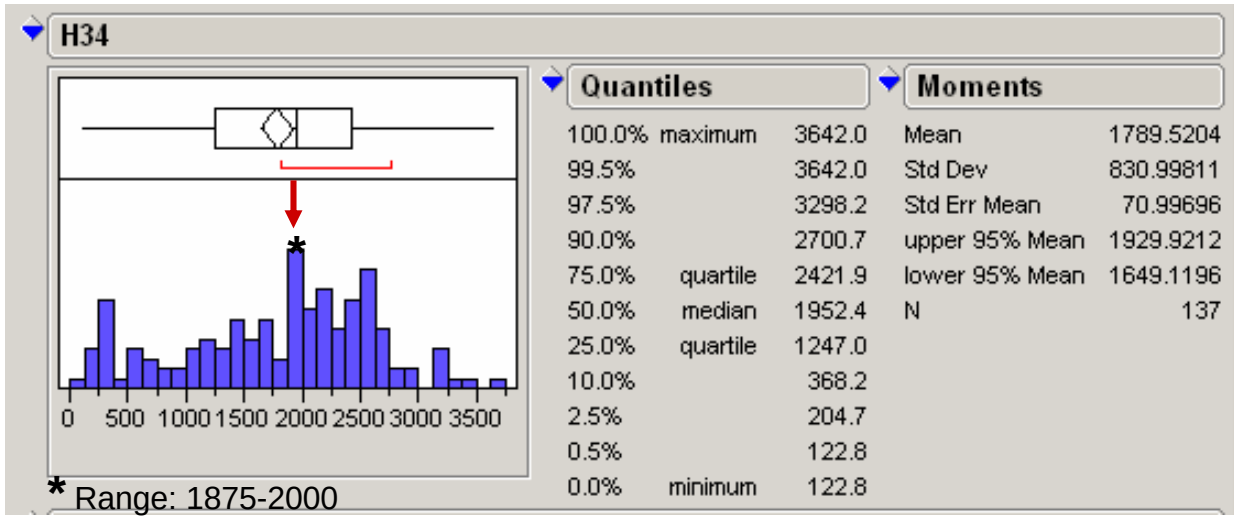
Tumor Mask



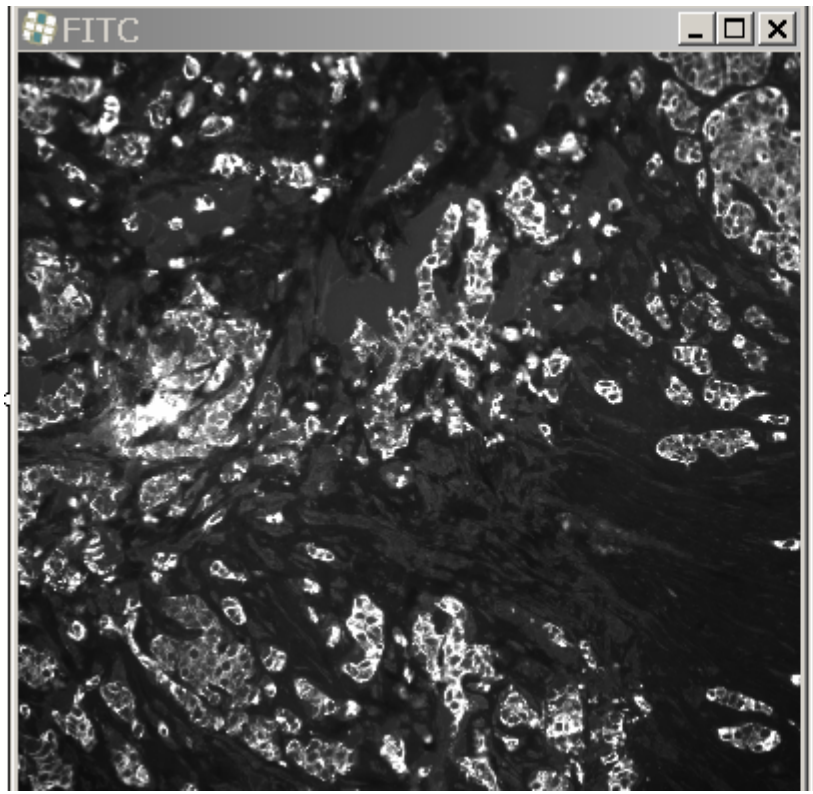
Target: MAP-tau

Case: H34.7

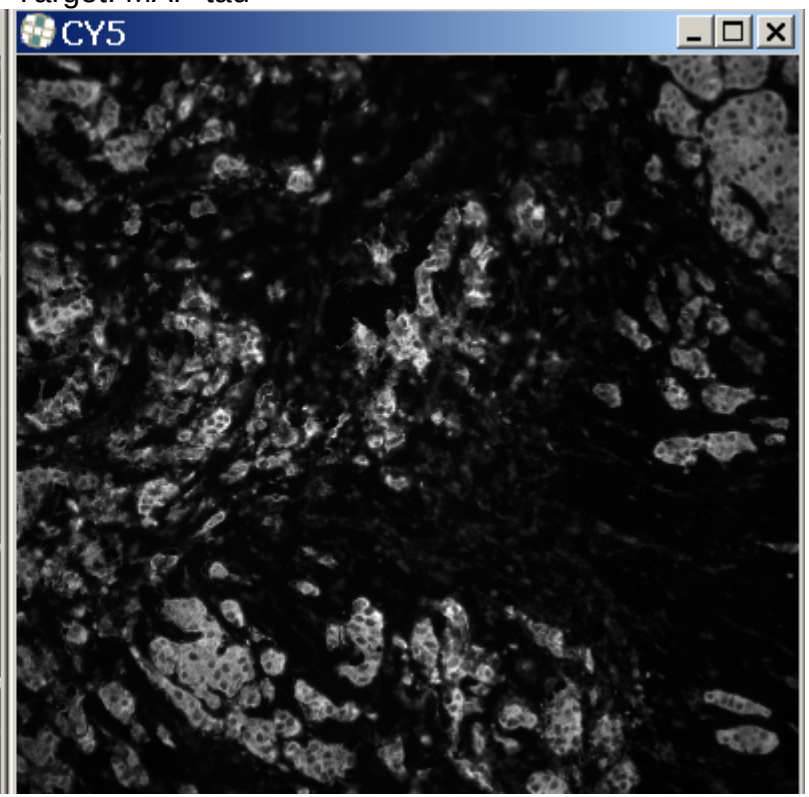
AQUA
Score: 1997.81



Tumor Mask

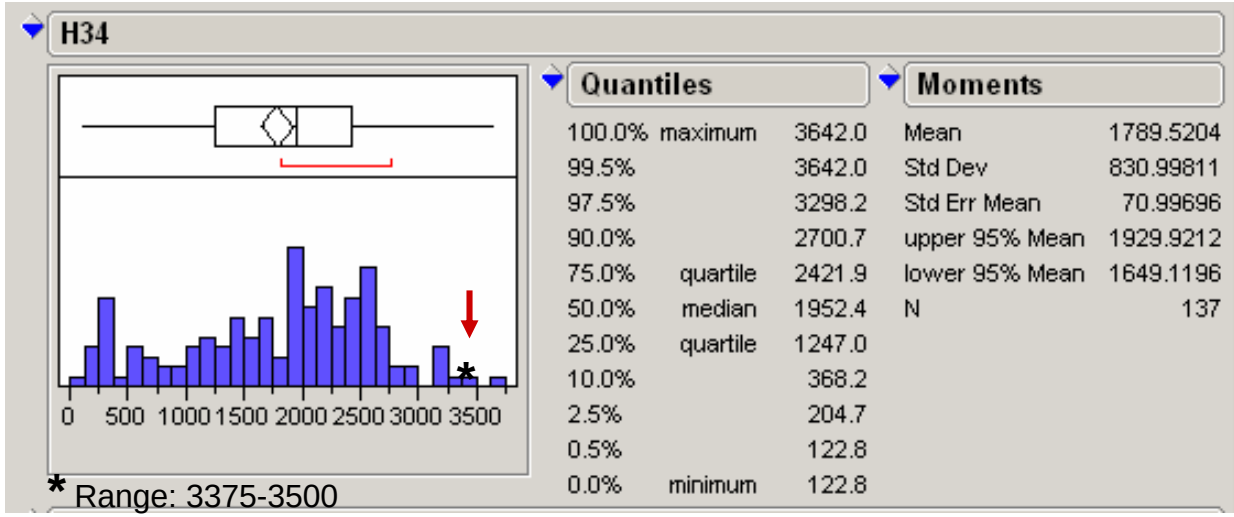


Target: MAP-tau

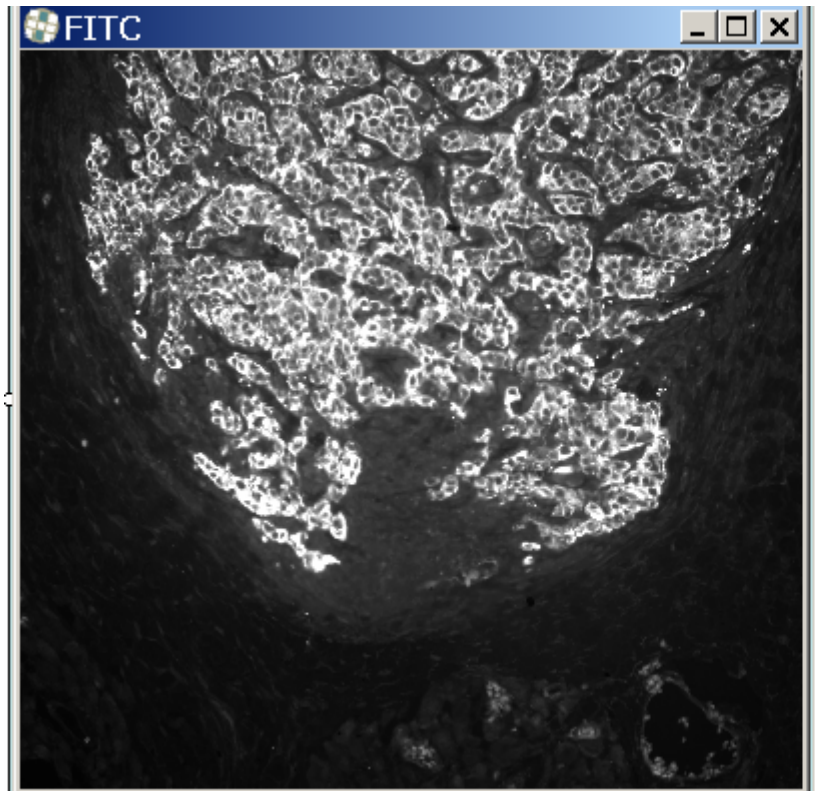


Case: H34.8

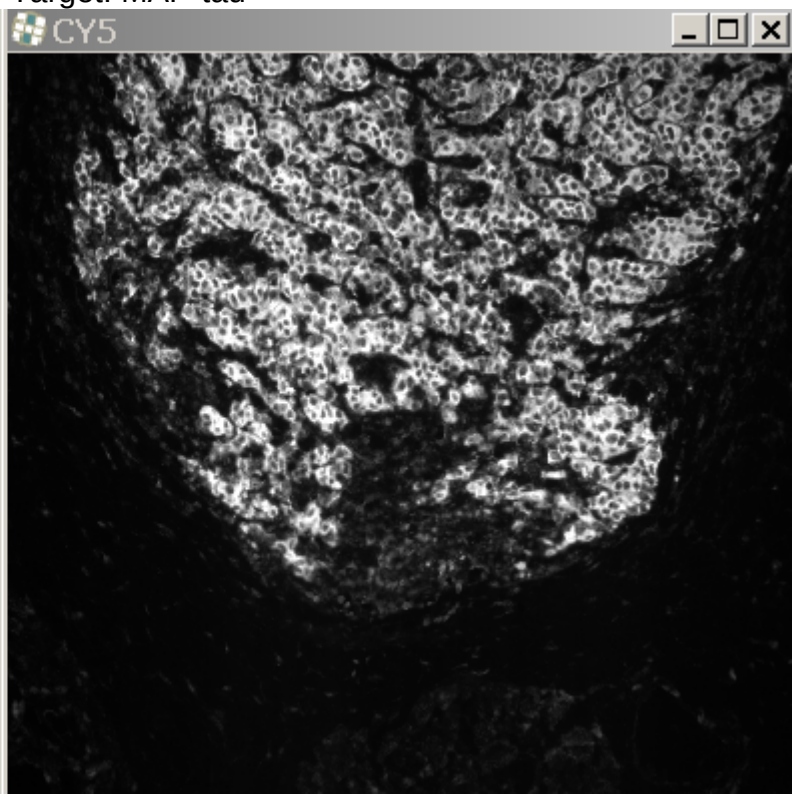
AQUA
Score: 3376.37



Tumor Mask

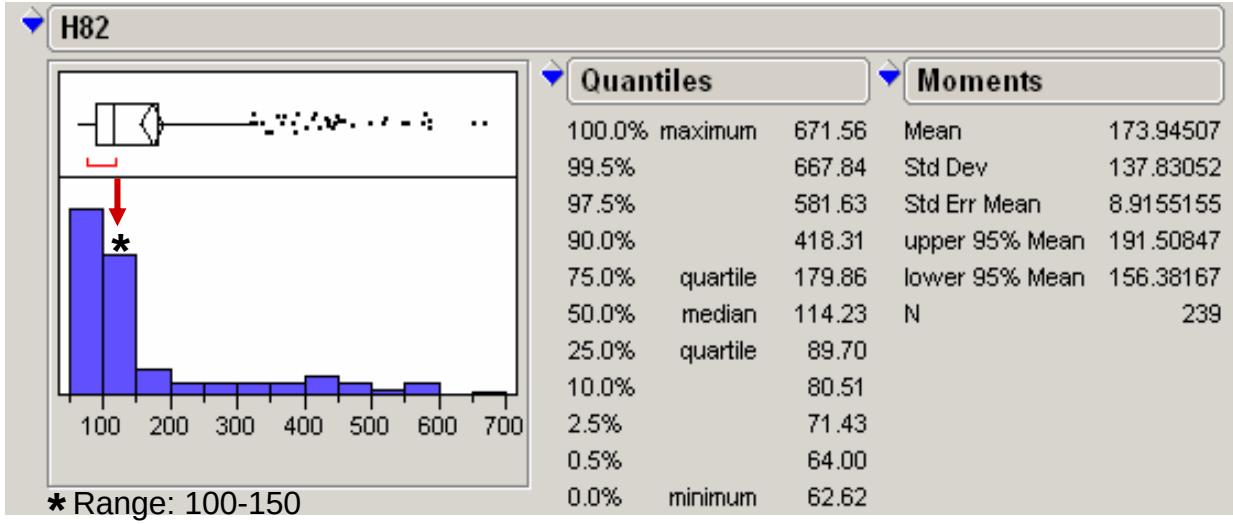


Target: MAP-tau



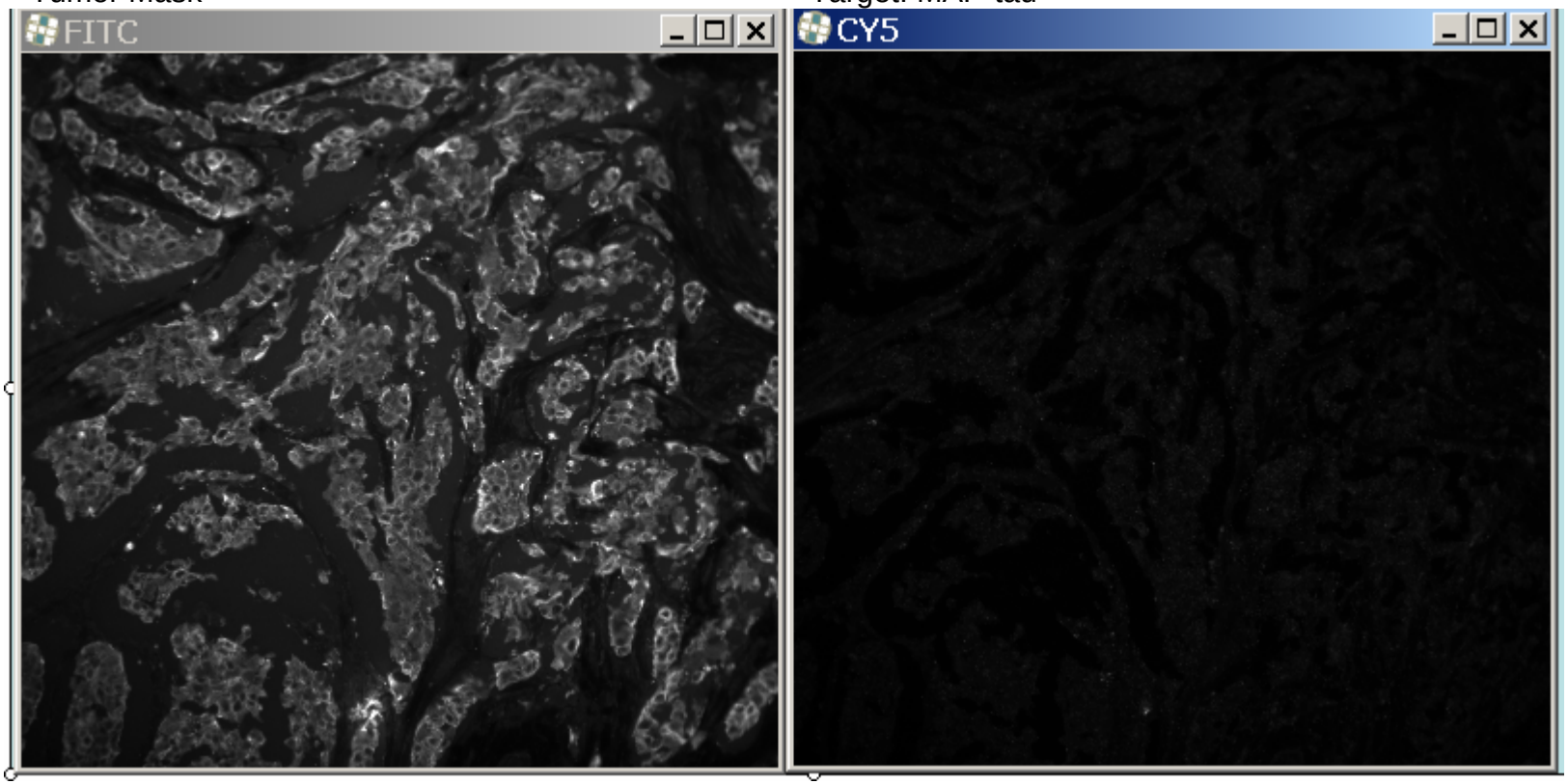
Case: H82.9

AQUA
Score: 105.16



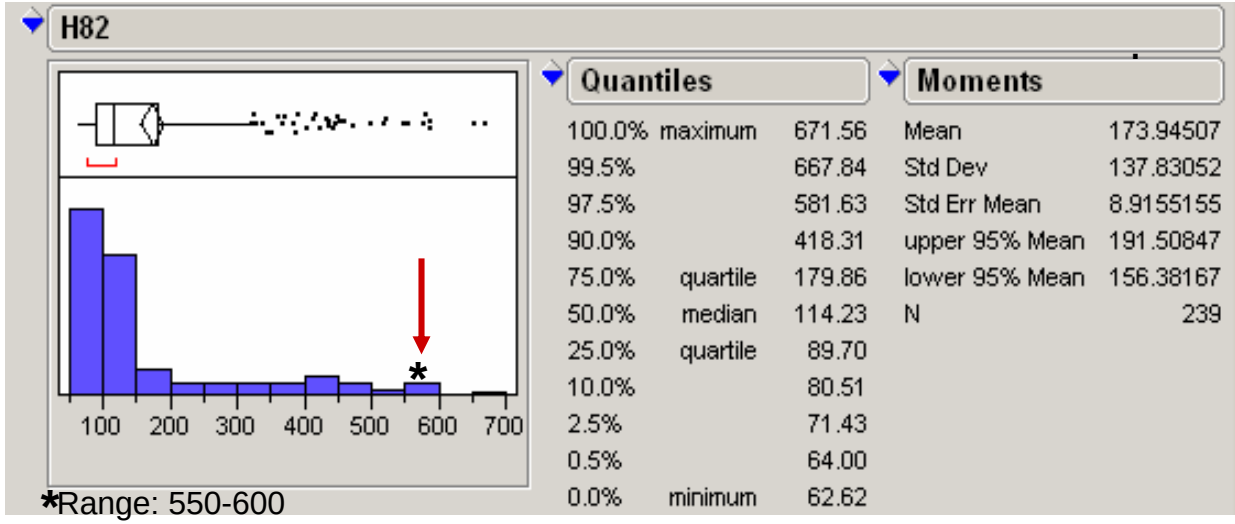
Tumor Mask

Target: MAP-tau



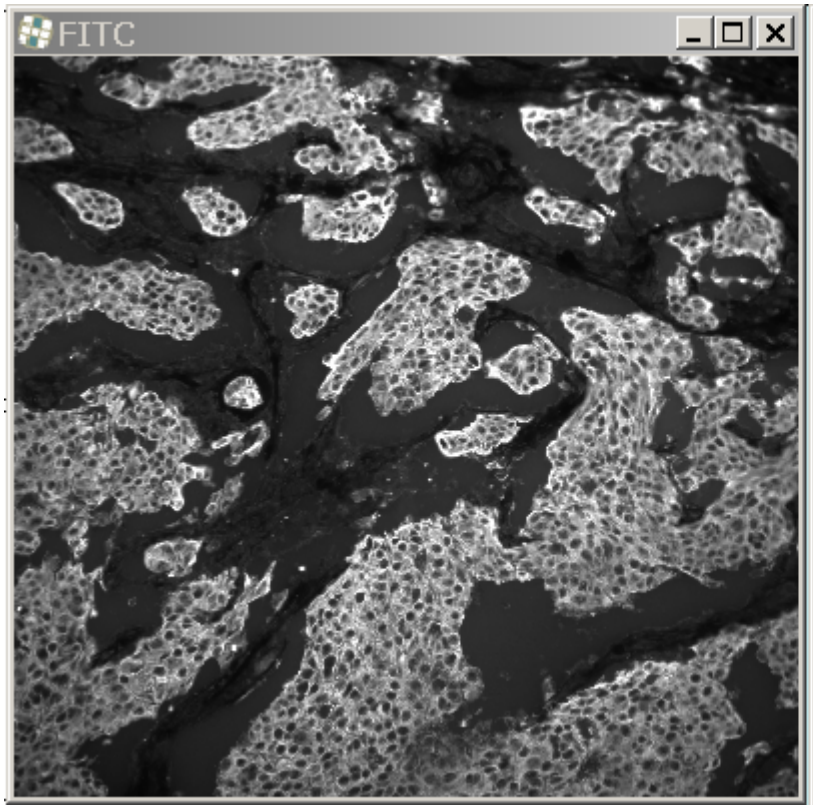
Case: H82.10

AQUA
Score: 587.53

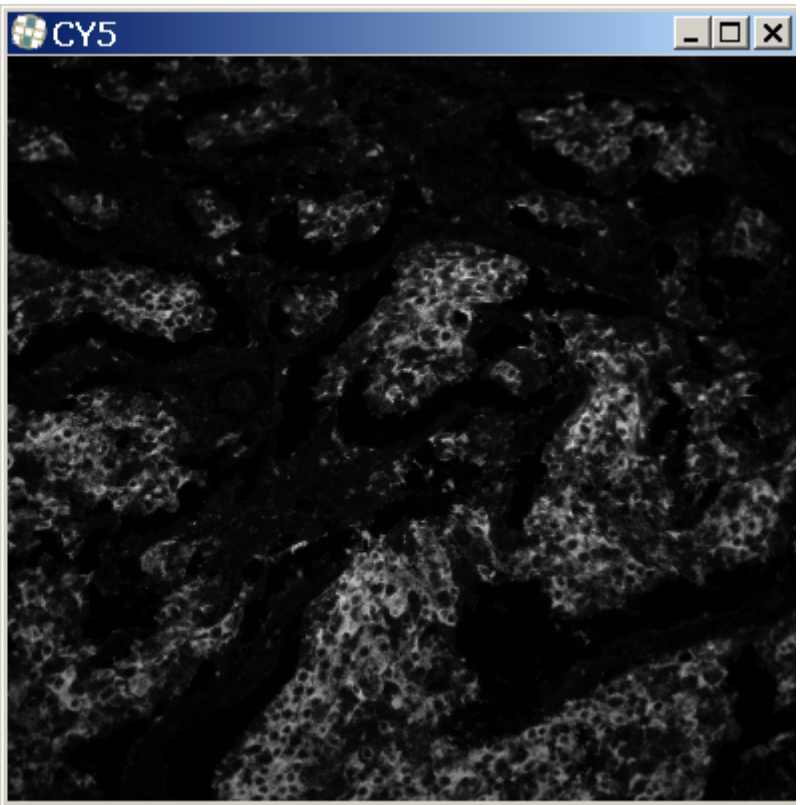


*Range: 550-600

Tumor Mask

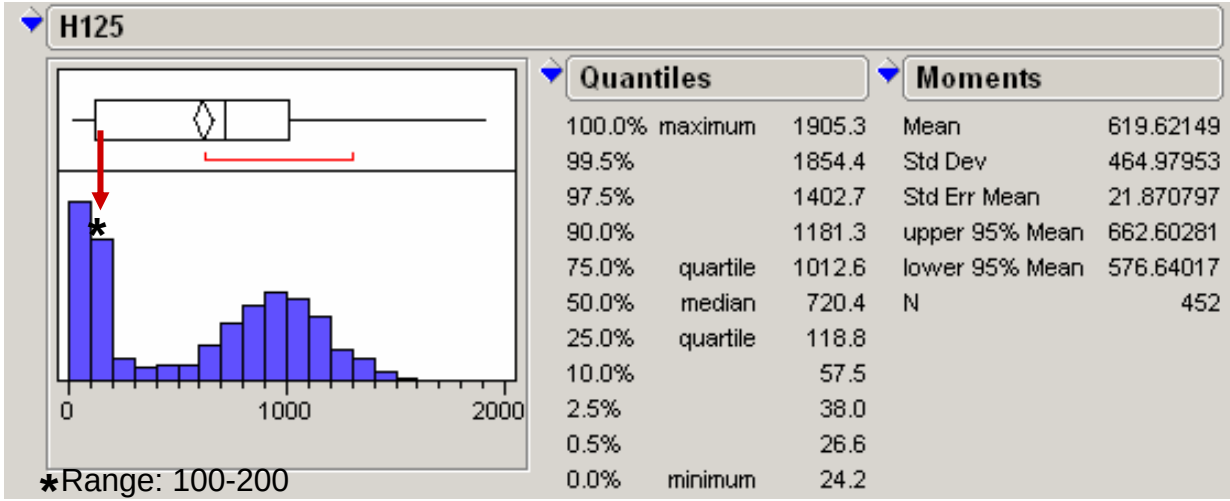


Target: MAP-tau



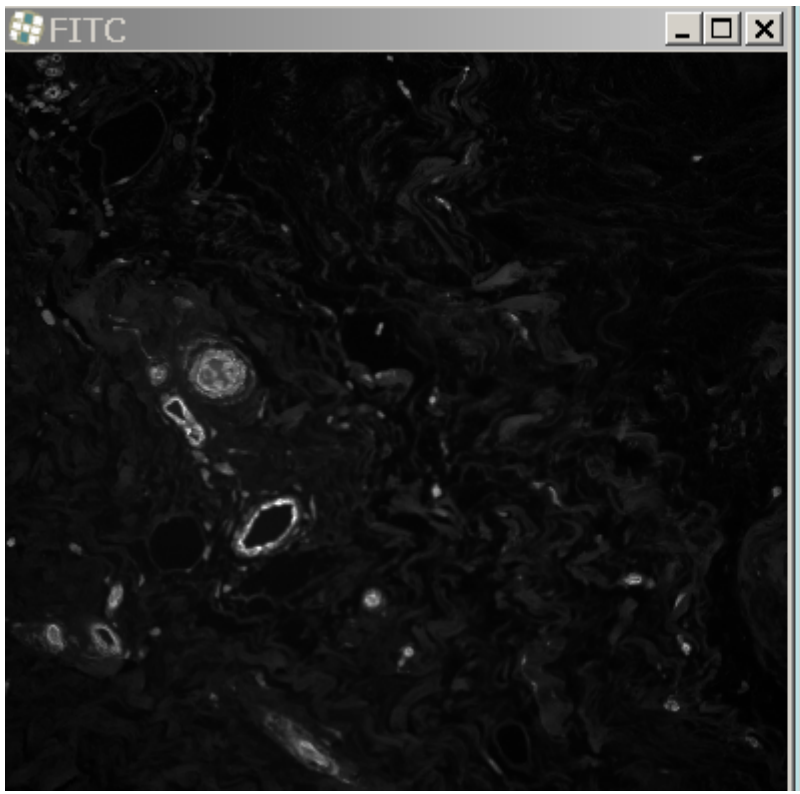
Case: H125.11

AQUA
Score: 106.12

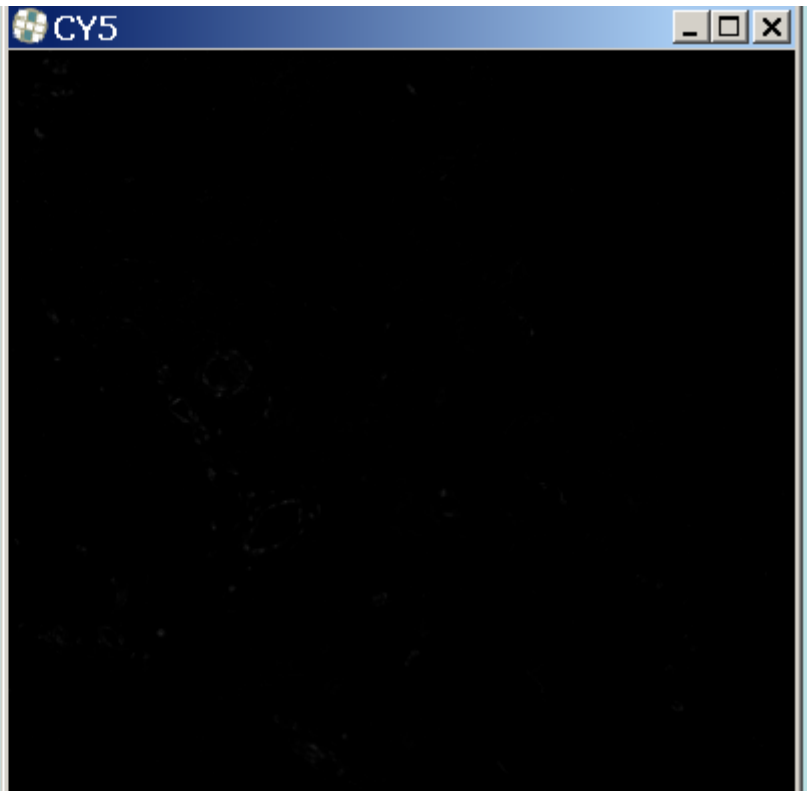


*Range: 100-200

Tumor Mask

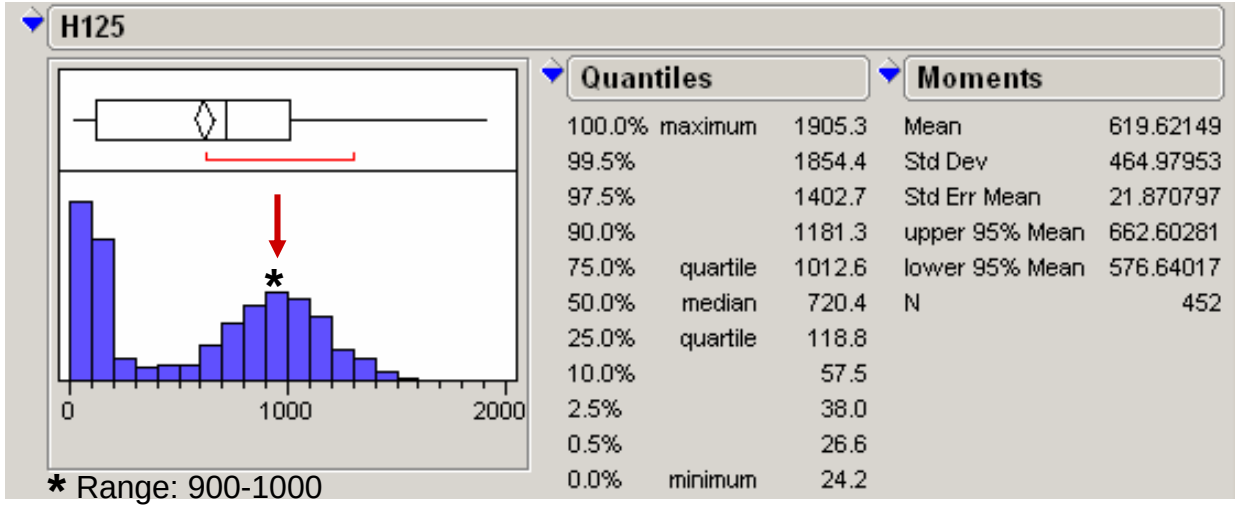


Target: MAP-tau

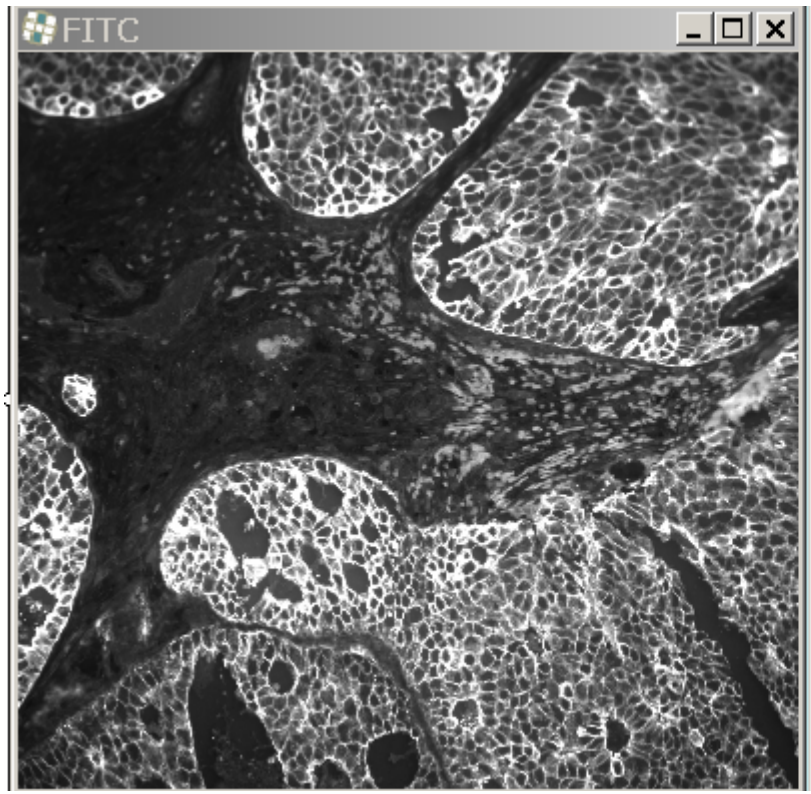


Case: H125.12

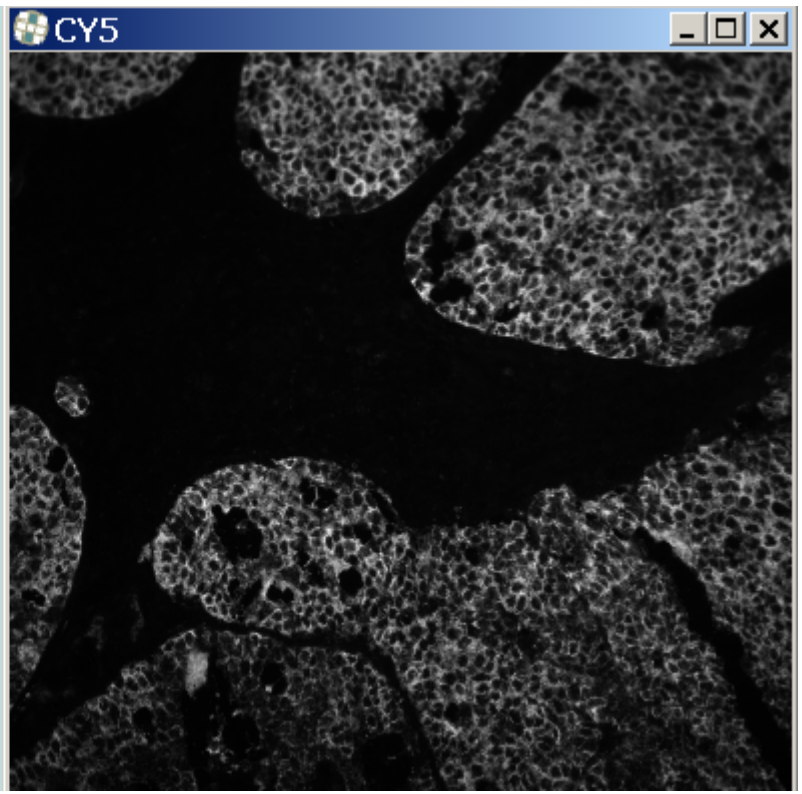
AQUA
Score: 973.06



Tumor Mask:

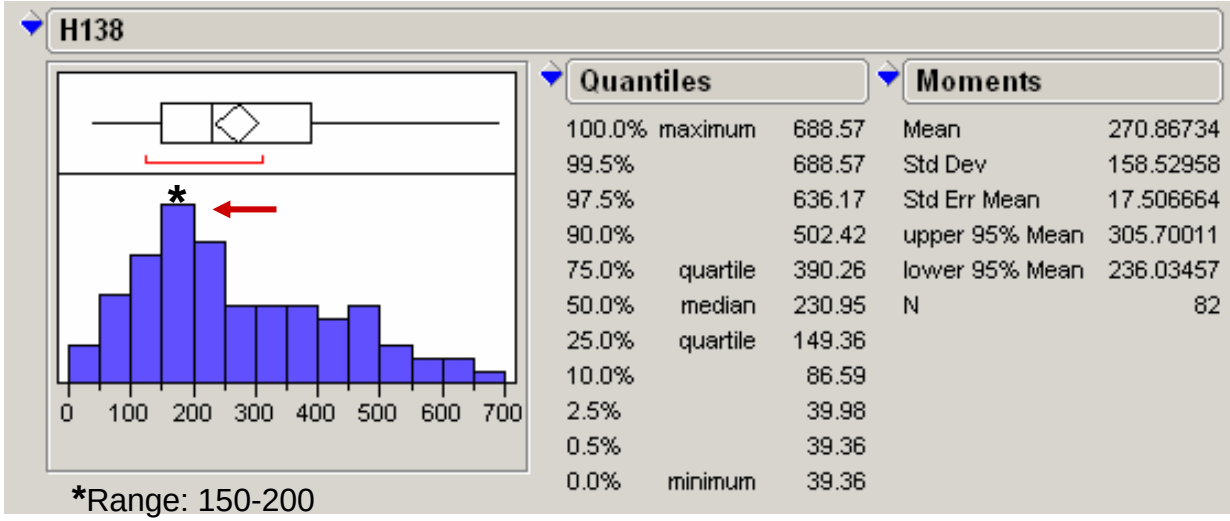


Target: MAP-tau



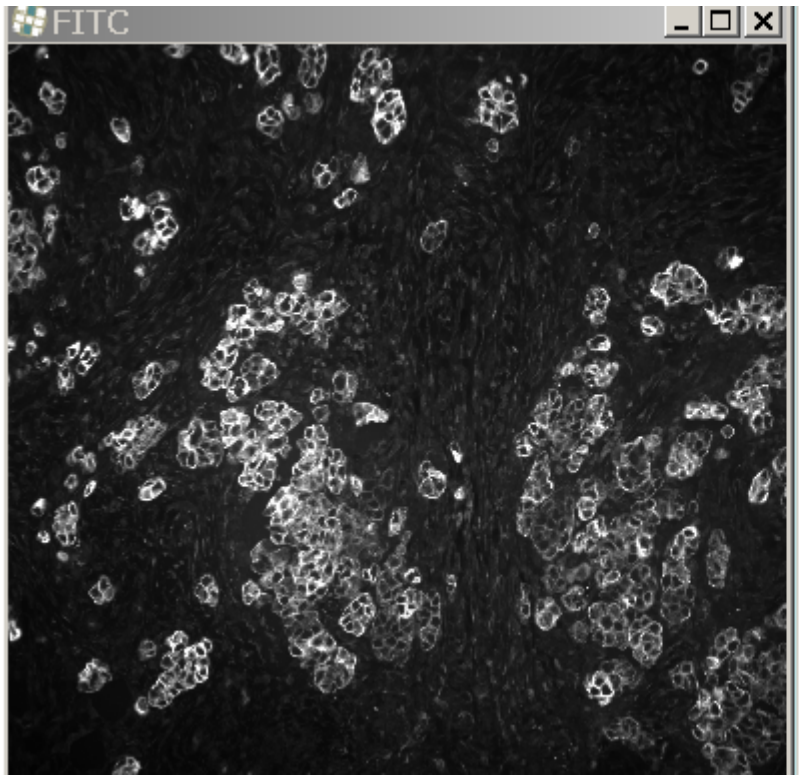
Case: H138.13

AQUA
Score: 173.79

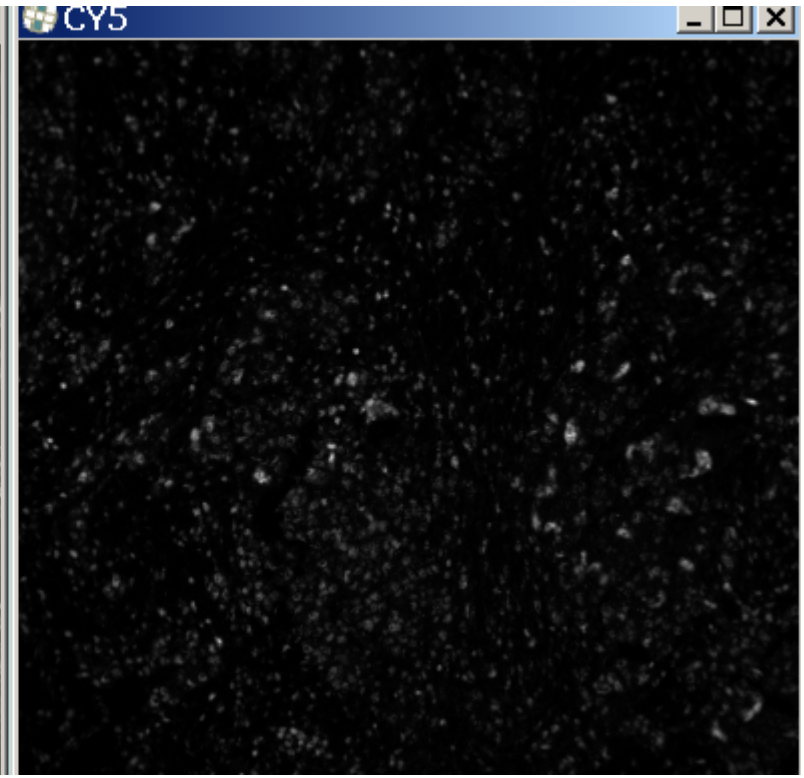


*Range: 150-200

Tumor Mask

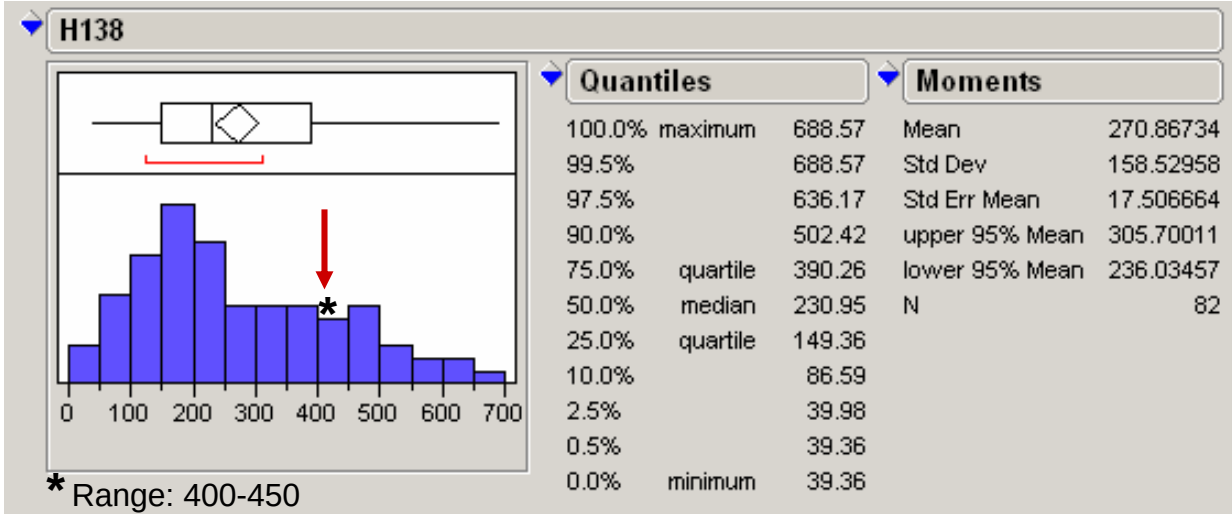


Target: MAP-tau

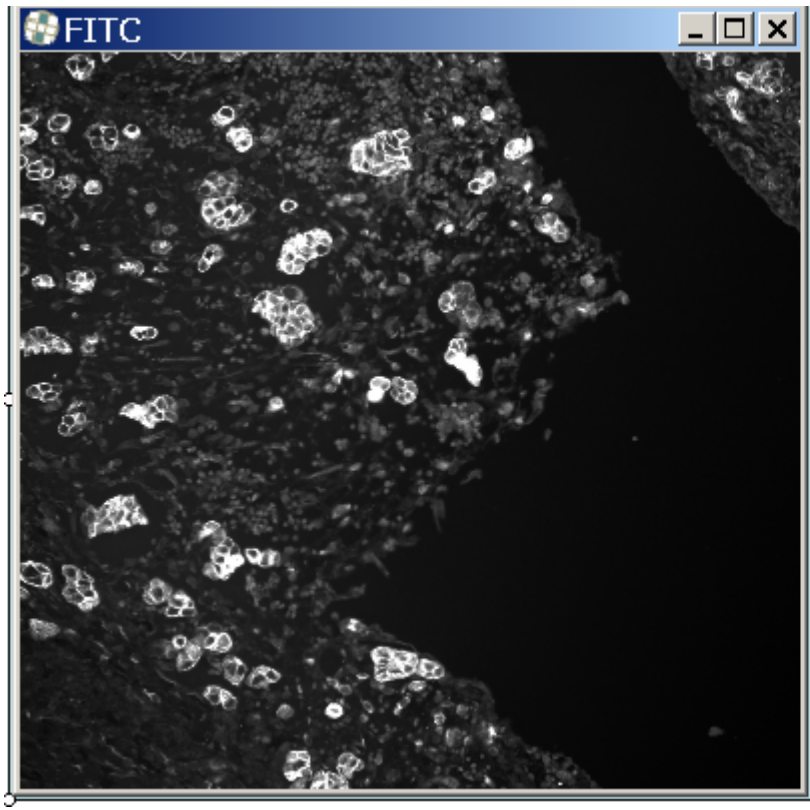


Case: H138.14

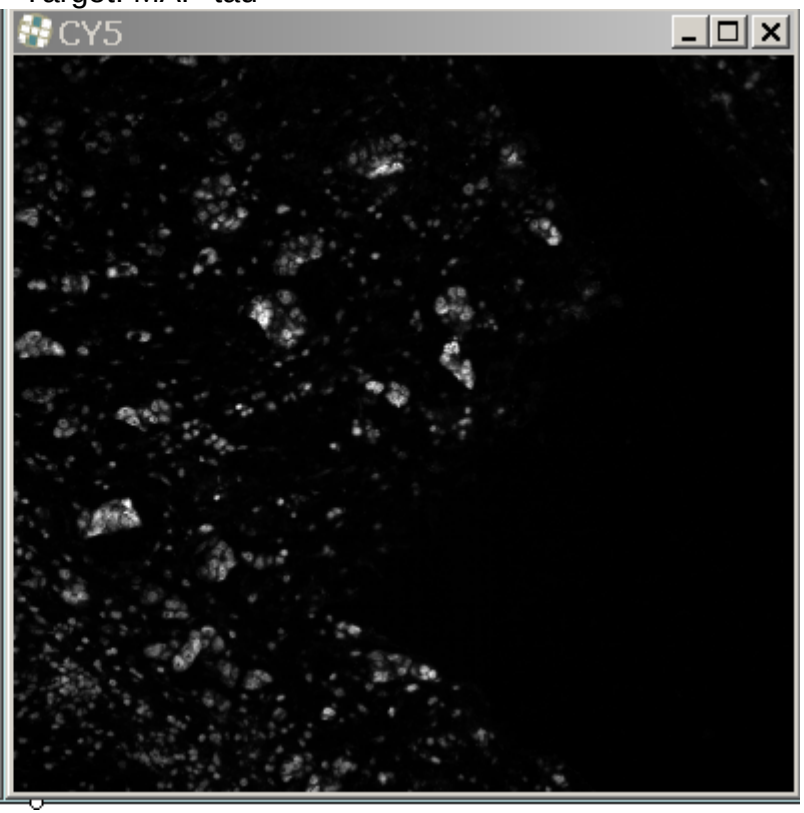
AQUA
Score: 426.82



Tumor Mask

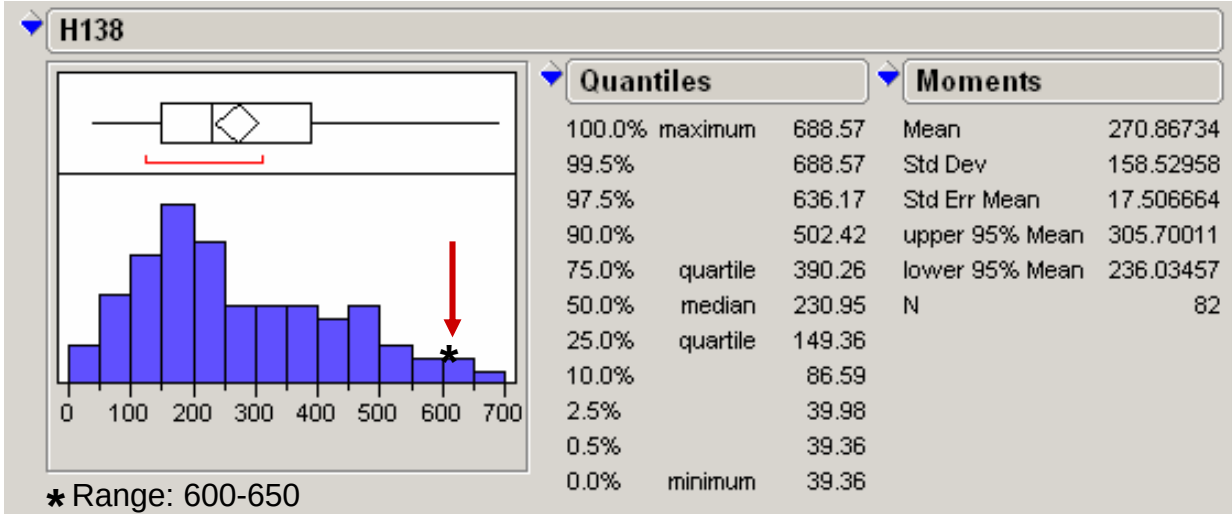


Target: MAP-tau

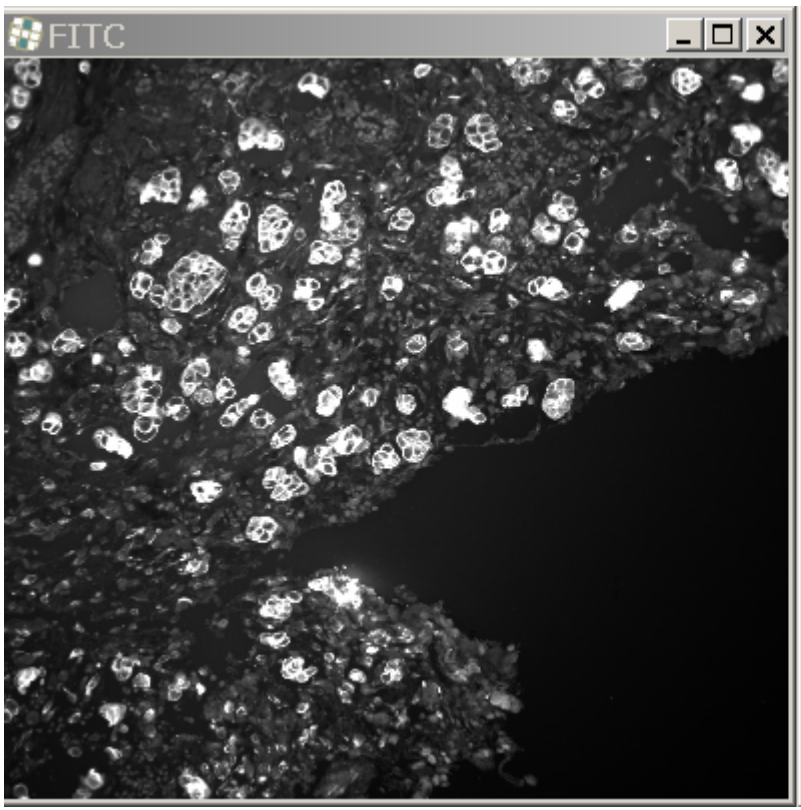


Case: H138.16

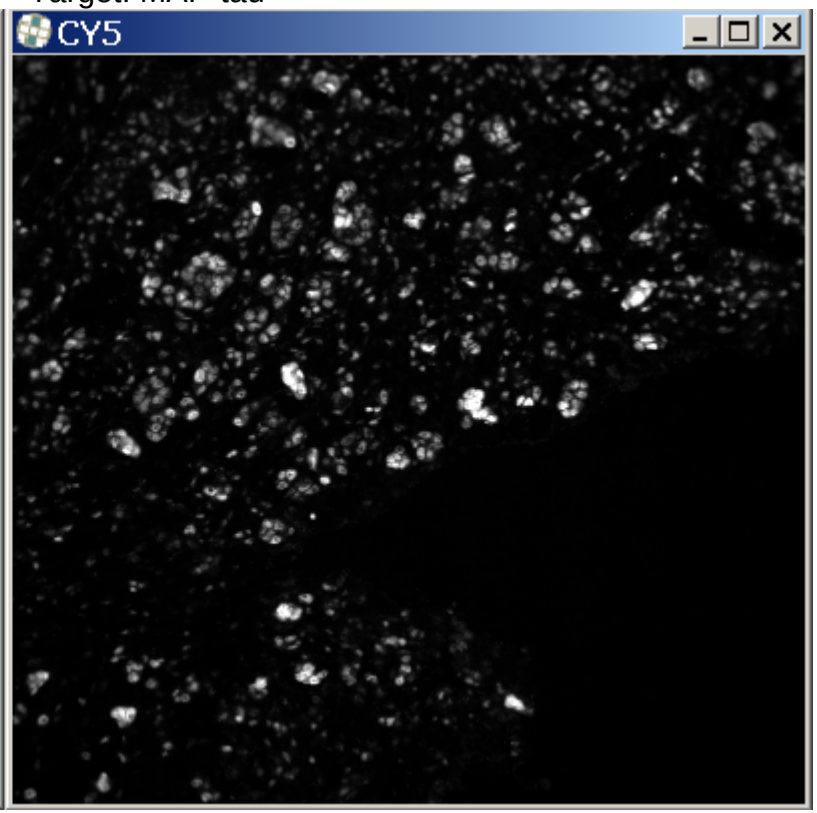
AQUA
Score: 619.50



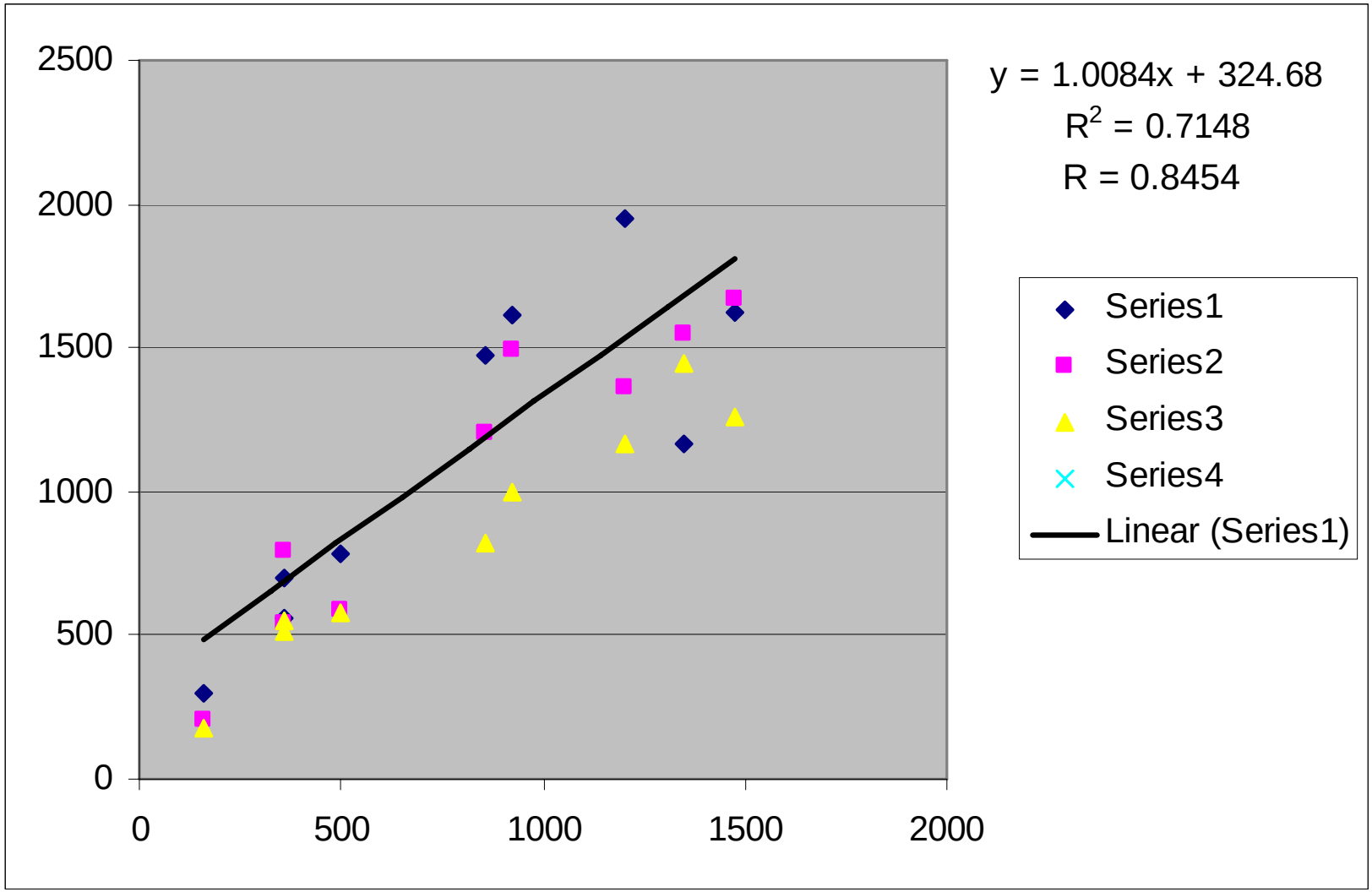
Tumor Mask

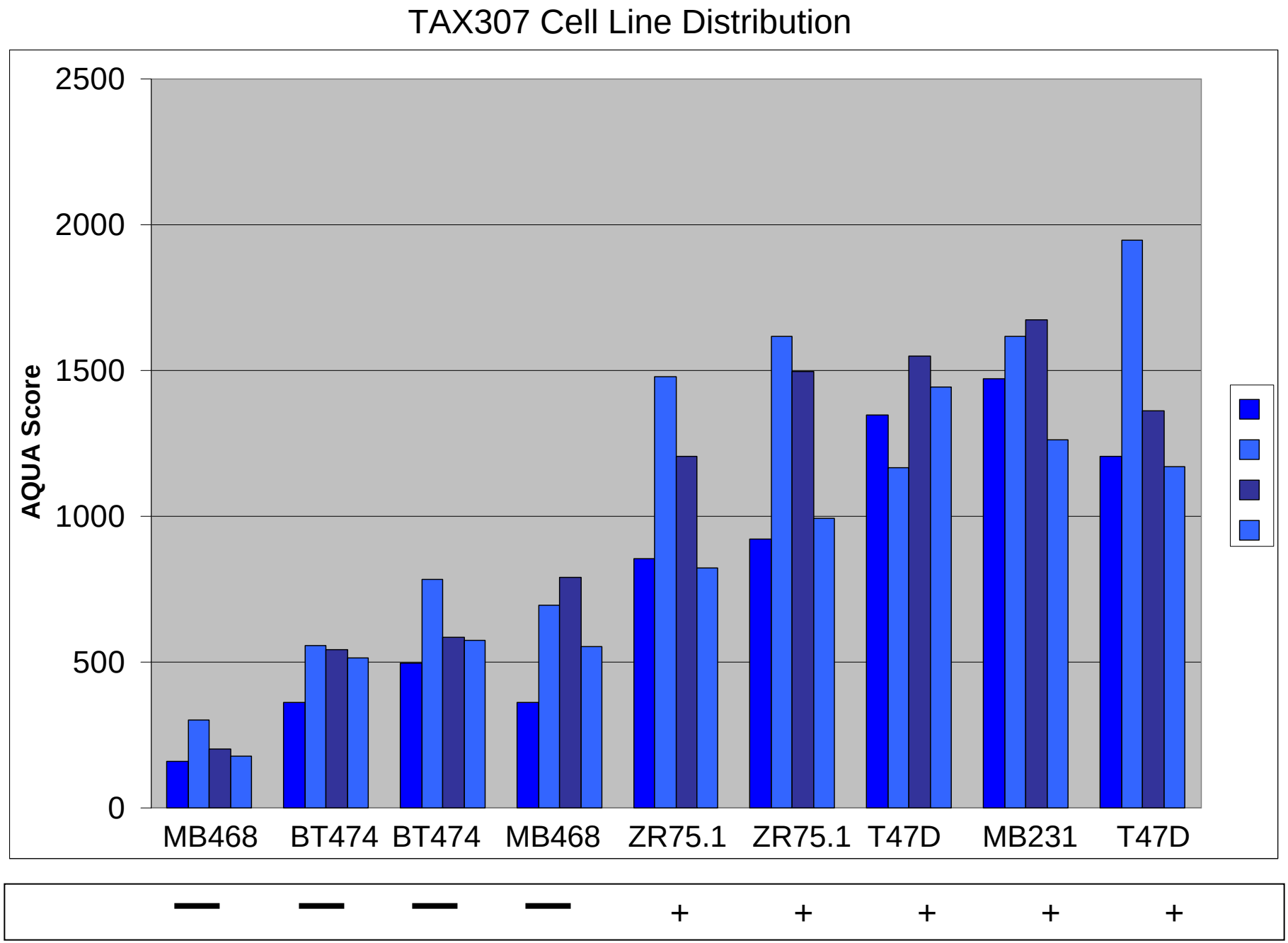


Target: MAP-tau

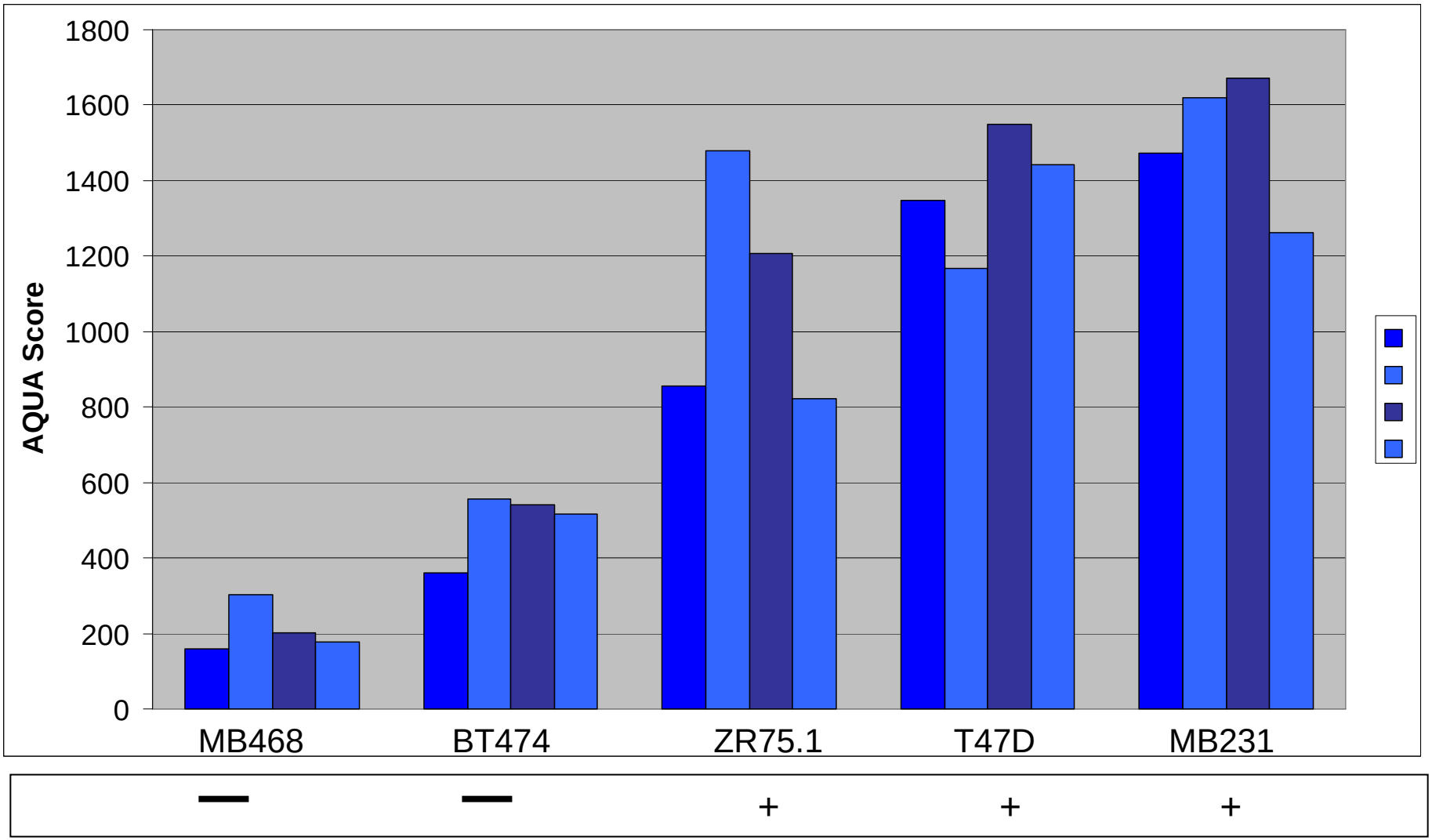
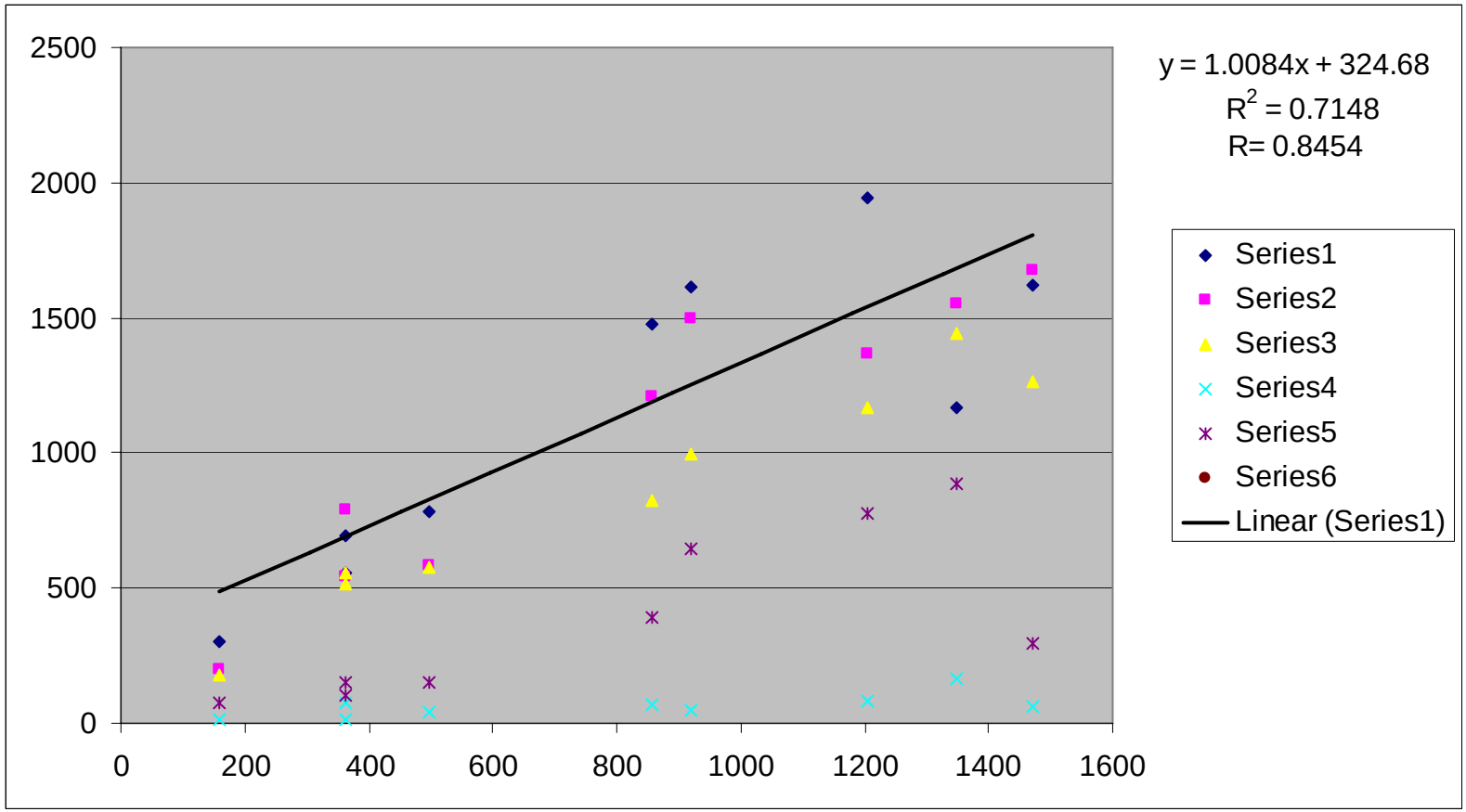


TAX307 Cell Line AQUA Score Regression for YTMA 94-1 Arrays




TAX307 Cell Line Distribution
AQUA Score
2500
2000
1500
1000
500
0
MB468
BT474
BT474
MB468
ZR75.1
ZR75.1
T47D
MB231
T47D
—
—
—
—
+
+
+
+
+

TAX307 Cell Line Distribution Grouped



TAX307 Cell Line AQUA Score Regression for YTMA 94-1 Arrays